BIOENERGETICS OF JUVENILE SALMON DURING THE SPRING OUTMIGRATION

Annual Report 1983

by

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Prepared for

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Project 82-11
Ronanld Morinaka, Project Officer
Contract DE-AI79-82BP35346

July 1985

ABSTRACT

Main stem reservoirs in the Columbia River Basin may have increased the energy demands of smolts during outmigration by prolonging migration and exposing smolts to seasonally rising water temperatures. A bioenergetic model for spring chinook salmon smolts (Oncorhynchus tshawytscha) is being developed to test these hypotheses.

Results have thus far indicated that the seaward migration can be separated into two distinct phases. Phase I can be described as a period of intense smolt development in which there was a post hatchery release surge in gill Na^+-K^+ ATPase activity, depletion of energy available in body lipids, and a concurrent decline in caloric density. Phase II was characterized by maintenence of smolt status in apparent anticipation of reaching the estuary. Phase II is the period most affected by impoundments and annual changes in water flow; the latter period will therefore be modeled in bioenergetic simulations.

Laboratory and field observations provided input parameters for the model and empirical data to verify model simulations. Total calories, caloric density, proximate body composition, ration, and caloric intake were determined in smolts as seaward migration progressed. The effect of swimming and starvation on energy reserves and seawater survival were determined in the laboratory. Fatty acid analysis indicated 03 neutral fatty acids influenced smolt development and seawater survival.

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ACKNOWLEDGEMENTS

We would like to thank Susan Gutenberger and Anita Allen for assistance in the laboratory; Wally Zaugg, National Marine Fisheries Service (NMFS), for gill-ATPase analyses; Sam Felton, University of Washington, for fatty acid analysis; Dick Anderson, Abernathy Salmon Culture Technology Center, U. S. Fish and Wildlife Service (USFWS), for helping to verify our method of proximate analysis; and Carl Schreck, Oregon State University, for allowing us the use of his laboratory.

We would like to also thank Teri Barilla U. S. Army Corps of Engineers (COE), and Tom Berggren and Gerry Bouck of Bonneville Power Administration for their constructive criticism and assistance.

We extend our sincere thanks to the following people for their help and cooperation in collecting fish from the Columbia River basin; Jim Mullen (Leavenworth Fisheries Assistance Office, USFWS); Virgil Mathias (Leavenworth NFH); Don Weitkamp (Farametrix Inc.); Steve Hayes (Chelan County PUD); Mike Dell (Douglas Co. PUD); Lee Ferguson, Tom Ruhle, and Brad Eby (NMFS and COE, McNary Dam); Rich Johnson and Dave Miller (NMFS, John Day Dam); Bill Muir, Lyle Galbreath, and Bob McConnel (NMFS, Bonneville Dam); Dick Ledgerwood and Earl Dawley (NMFS, Jones Beach); and most of all to the fish processing crews at McNary Dam, John Day Dam, Bonneville Dam, and Jones Beach.

We also wish to thank Daphne Erickson and Ginny Rehberg for typing the drafts.

INTRODUCTION

Mortality rates of juvenile chinook salmon (Oncorhynchus tshawytscha) during seaward migration have increased with impoundment of the Columbia and Snake rivers. Causes of mortality during migration through impoundments have been attributed to predation, residualism, and other factors associated with reservoir habitats. We hypothesize that the increased energy demands of prolonged seaward migration and/or additional energy expended while migrating through impoundments reduces the smolt's energetic condition or health and subsequently survival rates.

Substantial losses in energy reserves (e.g. lipids) take place during seaward migration. Part of these losses can be attributed to changes associated with smoltification, however, additional energy may be required by up-river smolts due to: 1) migration periods that have increased as much as 100% because of impoundments, 2) increases in temperatures experienced during seasonal delay of outmigration, 3) additional energy expended on active swimming in reservoirs, and 4) limited food consumption, especially by smolts released from hatcheries. These additional energy requirements could result in a negative energy budget (i.e. metabolic requirements greater than energy intake) for smolts during the seaward migration. An extended migration with a negative energy budget would deplete energy reserves of migrating salmon, and possibly decrease survival.

This study will formulate an energy budget for migrating smolts using data from the literature and experimental results from the

laboratory and the Columbia River basin. A computer model will be prepared from this information to simulate the energy budgets of migrating spring chinook salmon smolts as a function of water temperature and flow regimes in the Columbia River.

Approach

A physiological approach has been chosen to address the bioenergetics of migrating spring chinook salmon smolts, with the energy budget divided into the following components,

$$C = \Delta B + R + U + F$$

where:

C = daily caloric intake, the caloric value of food consumed (cal/day);

ΔB = daily change in total caloric value of the smolt, resulting from food consumption in excess (+ ΔB) or deficient (- ΔB) of metabolic needs (B is the total caloric value of the average fish);

R = respiration, the total caloric requirement of metabolism (respiration can be partitioned into standard metabolism, R_s; specific dynamic action, R_{sda}; and energy released during swimming activity, R_a);

U = urinary loss, the caloric value of the excretory products; and

F = fecal loss, the caloric value of fecal waste.

The model is composed of a main program to simulate the energy budgets of chinook salmon smolts and several subroutines to calculate input variables as shown in the flowchart (Fig. 1). The subroutines use the abiotic input variables to estimate the location of the fish during the seaward migration. The location, date, and flow regime are used to

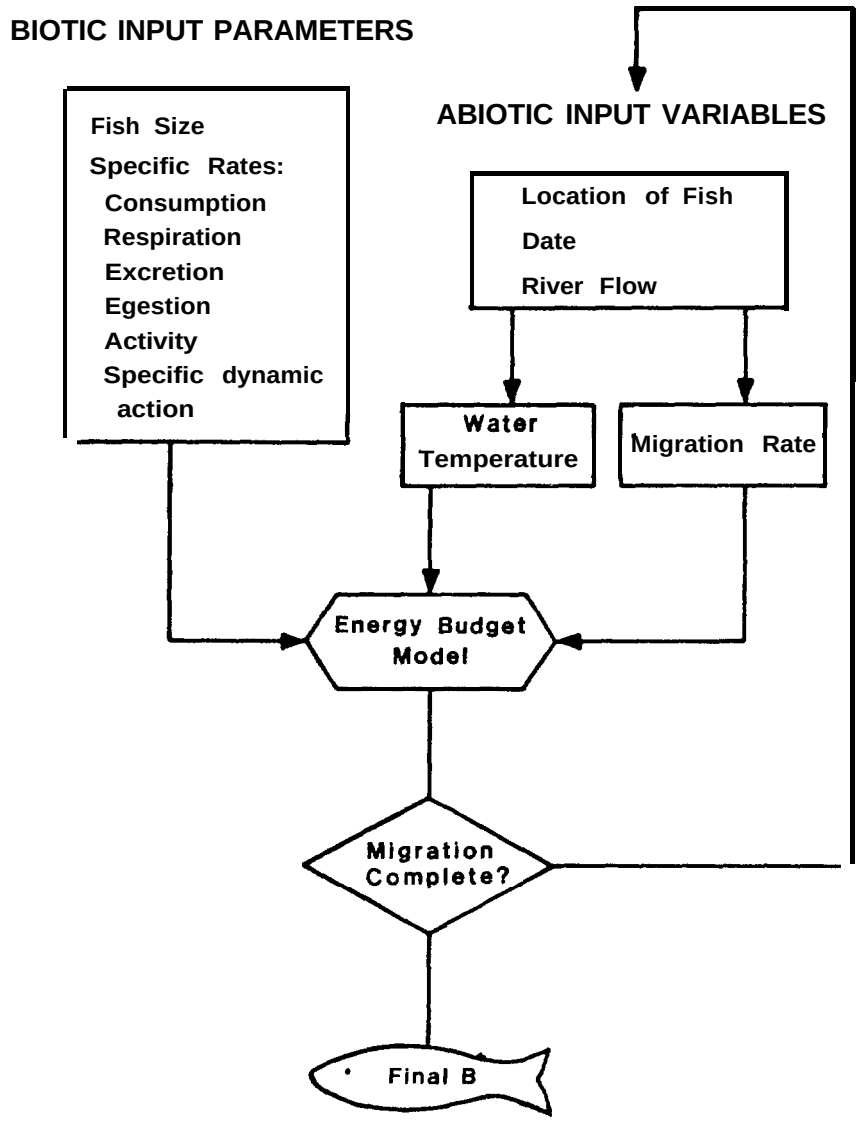

Figure 1. Flowchart of the bioenergetics model for spring chinook salmon smolts during seaward migration.

estimate ambient water temperature for each day of migration. The model uses the physiological (biotic) parameters with selected environmental (abiotic) factors to simulate changes in total caloric value of fish (AB) on a daily basis.

The model will be applied to spring chinook salmon smolts during their seaward migration to determine which combinations or levels of abiotic factors impact smolt energy budgets. Model simulations will enable water and fisheries managers to identify river flow regimes and water temperatures that are most likely to reduce smolt survival.

Objectives: Calendar Year 1983

1. Estimate mean total caloric value (B) of spring chinook salmon smolts at selected locations during seaward migration.
2. Estimate daily caloric intake (Cal/fish/day) of spring chinook salmon smolts during seaward migration.
3. Determine effect of swimming activity (Ra) and food consumption (C) by smolts on growth ($\triangle B$) and survival in seawater challenge.
4. Determine effects of starvation and lipid loss on survival of smolts in seawater.

Addressing the first objective was necessary to estimate mean total caloric value of smolts during migration for later comparison with model simulations. Daily caloric intake (objective 2) was estimated for use as input in the consumption component of the model. Laboratory experiments were necessary to address objective three, since swimming activity and food consumption were difficult to measure under

field conditions. Results were used to determine if assumptions concerning swimming activity (Ra of model) and food consumption (C of model) were reasonable for model simulations. Results for objective four were used to interpret measurements of lipids and food consumption of fish collected at selected locations.

TOTAL CALORIC VALUE - B

Objective 1: Estimate mean total caloric value (B) of spring chinook salmon smolts at selected locations during seaward migration.

Introduction

The caloric values of smolts at selected locations during the seaward migration are required for later validation of the model simulations. Validation will involve comparison of model simulations of smolt energetics with data collected from the Columbia River basin. Comparing observations with simulations will enable us to determine how well the model represents actual changes in the energy budgets of smolts during seaward migration.

The energy reserves of smolts are altered during seaward migration by the physiological state of the smolt as well as the environmental conditions encountered. Physiological, morphological, and behavioral changes associated with smoltification cause an increase in the metabolic demand for energy (Baraduc and Fontaine 1955; Folmar and Dickhoff 1980; Wedemeyer et al. 1980). The increased metabolic rates are reflected by increased levels of circulating hormones (thyroxine and cortisol) and increased enzyme activities (Na+-K+ ATPase). Morphological changes that require energy involve elaboration of tissue resulting in changes in fin and body shape (Gorbman et al.

1982; Winans 1984). In addition, behavioral changes cause increased swimming activity and a more pronounced response to stress during smoltification (Congleton et al. 1984). Since smoltification and migration occur at approximately the same time, their effects on energy reserves of smolts cannot be separated.

Energy reserves were estimated by determining caloric values of smolts collected at selected locations along the Columbia River. Energy reserves in constituents such as glycogen or glucose were disregarded because the amount of energy in these constituents are relatively small (Webb 1975; Caulton and Bursell 1983). Only the major body constituents such as water, protein, lipid and ash, were determined for the calculation of total caloric values.

Methods

Fish sampled to estimate energy reserves were marked for a study described by Mckenzie et al. (1984) on the survival of downstream migrant spring chinook salmon. Spring chinook salmon of the Carson NFH stock were reared at Leavenworth National Fish Hatchery (NFH) and branded and released at the mouth of the Methow River, Rkm 838. The groups of fish branded IL-1 and IZ-2 were released on April 22 and May 4, 1983, respectively. Each branded group contained approximately 50,000 fish. Ten fish were sampled from each group at Leavenworth NFH the day before release, and subsequently at sampling facilities at Rocky Reach Dam (Rkm 763), Priest Rapids Dam (Rkm 639), McNary Dam, (Rkm 467), Bonneville Dam ((Rkm 232), and Jones Beach (Rkm 75) (Fig. 2).

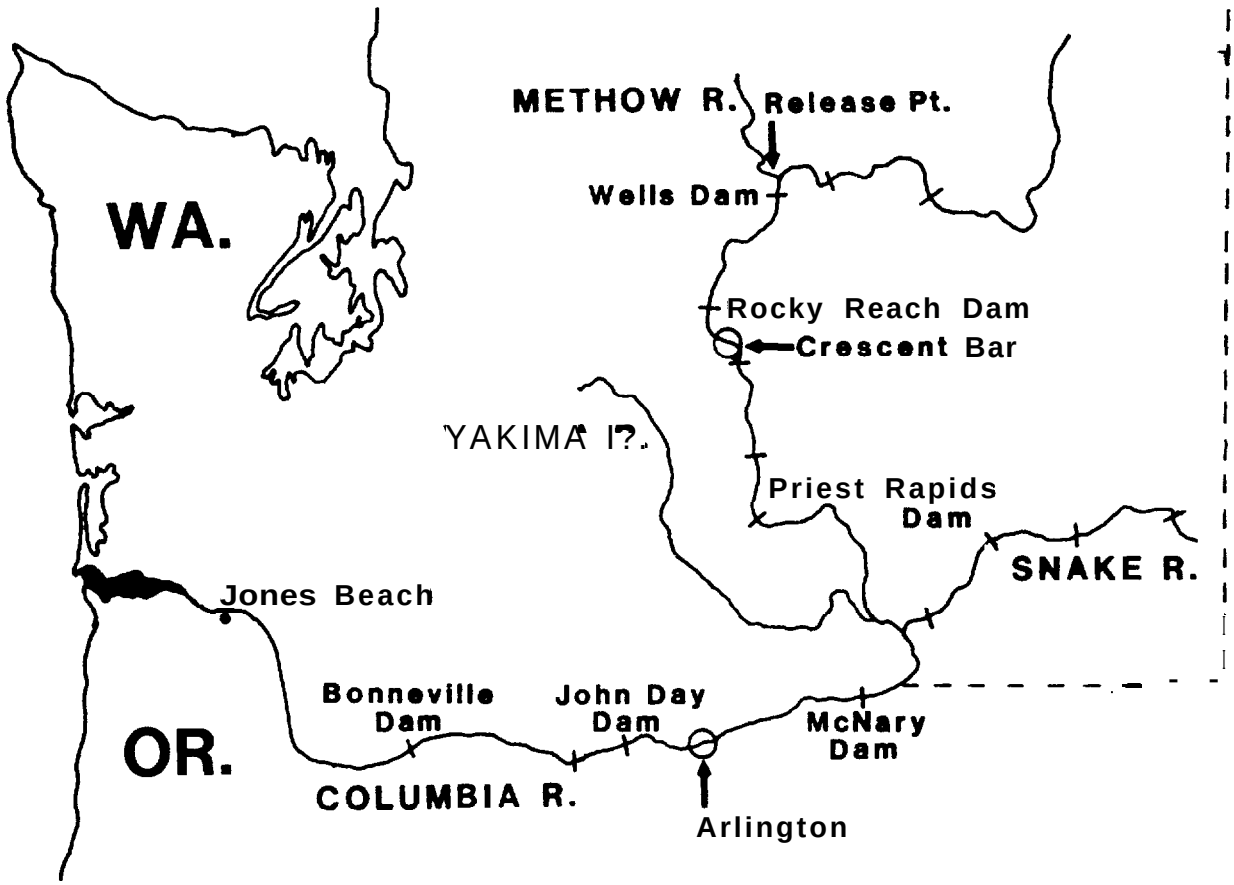

Figure 2. Map of Columbia River basin.

Samples were frozen and returned to the laboratory for analysis of lipid, water, ash, and protein content, hereafter collectively referred to as body constituents. Analysis of body constituents for fish collected during 1983 were limited to a single release group branded IZ-2 because all groups released in 1982 lost lipid at similar rates.

Each fish was homogenized with a micro-blender, and lipids were extracted from a 2 g subsample using the method of Bligh and Dyer (1959) as modified by Hanson and Olley (1963). A 3 g subsample of homogenized fish was dried for 48 hours at 65 C to determine water content, then placed in a muffle furnace for 3 hours at 600 C to determine ash content (Paine 1971). Total protein was estimated by subtracting estimated total weight of lipid and ash from total dry weight of fish. Caloric values of fish were calculated by multiplying the weight of each constituent by its caloric equivalent, 9.3 kcal/g for lipid and 5.65 kcal/g for protein (Brett and Groves 1979). The caloric density was calculated by dividing the total caloric value by the wet weight of the fish and a mean caloric value was determined for the 10 fish sampled at each location.

Gill Na^+-K^+ ATPase activity was used as an indicator of smoltification. Samples of ten fish were collected from each release group at the locations described above. Fish were anesthetized in a 100 mg/litre solution of MS-222, then killed by a sharp blow to the head. Gill filaments used for determination of Na^+-K^+ ATPase activity were trimmed from the gill arch and washed into test tubes with 2 ml of sucrose ethylenediamine imidazole and frozen immediately on dry ice. Na^+-K^+ ATPase activity was determined by the method of Zaugg (1982).

Analysis of variance was used to determine if significant differences existed between and within individual data sets. If differences were significant a Newman-Keuls multiple comparison test was used to identify differences between individual means. Lipid data, expressed in percentage, was transformed using an arcsine transformation prior to statistical analysis (Sokal and Rolf 1981).

Results and Discussion

Mean caloric values of spring chinook salmon smolts did not change significantly during migration from the release location near Pateros (Rkm 838) to Jones Beach (Rkm 75). The results were consistent with 1982 indicating that net growth (AB) was zero for migrating smolts ($P>0.05$, Appendix 1A; Fig. 3).

Although total caloric value did not change significantly during migration, some body constituents, important in the determination of total caloric value, changed substantially. Figure 4 illustrates changes in the body constituents during downstream migration in 1983. Percent body protein and ash showed only small differences among sampling locations. Body lipids declined about 65%, from 4.3% at the release point to 1.4% at McNary Dam ($P<0.05$, Appendix 1B). Lipids remained relatively constant at about 1.4% downstream from McNary Dam. Percent body water was inversely related to percent body lipid and increased as lipids were depleted.

The 1.5-2.0% body lipid level is about the lowest percentage of lipid observed in most species of fish, even under conditions of

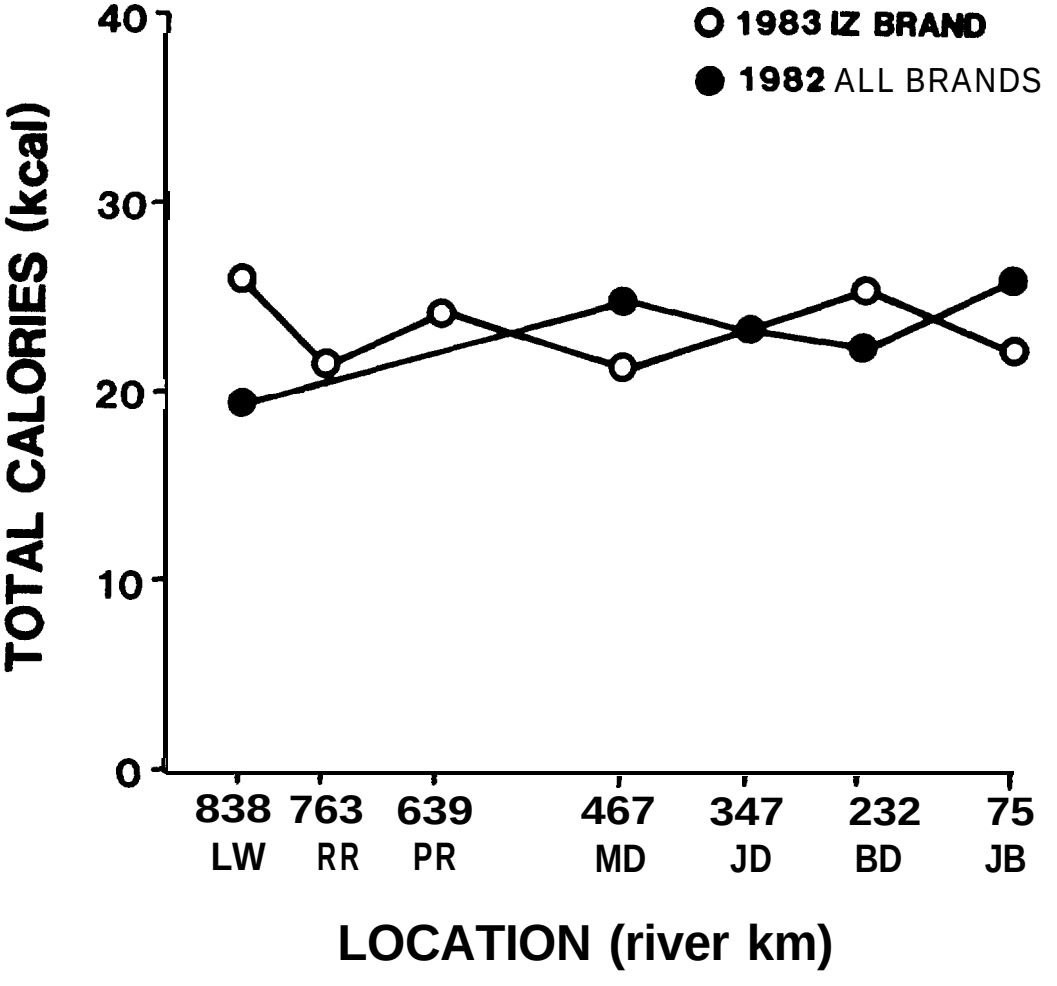

Figure 3. Mean total caloric value of marked spring chinook salmon smolts sampled in 1982 and 1983 at Leavenworth NFH (LW), Rocky Reach Dam (RR), Priest Rapids Dam (PR), McNary Dam (MD), John Day Dam (JD), Bonneville Dam (BD), and Jones Beach (JB).

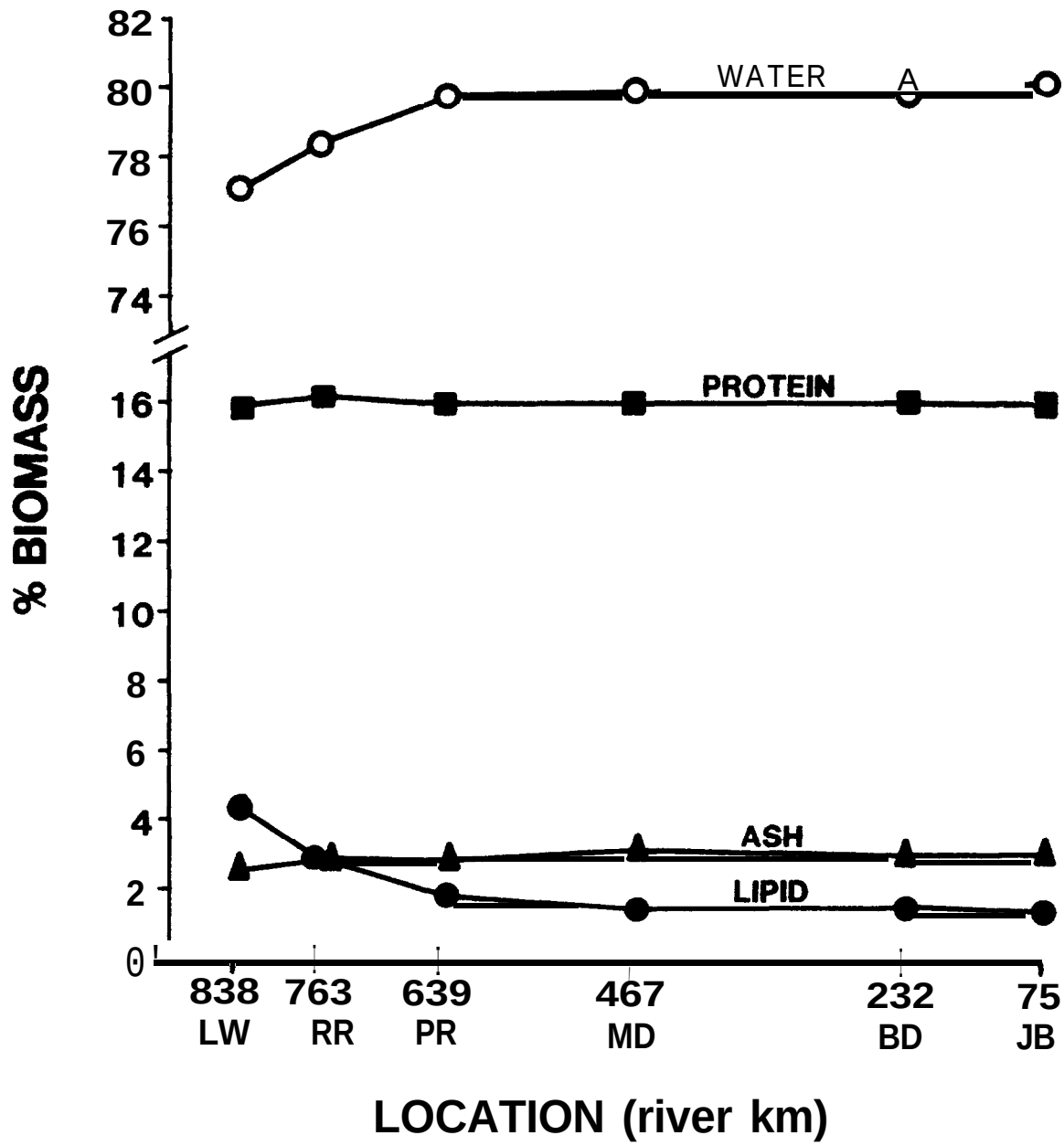

Figure 4. Percent composition of body constituents in spring chinook salmon smolts collected at Leavenworth NFH (LW), Rocky Reach Dam (RR), Priest Rapids Dam (PR), McNary Dam (MD), Bonneville Dam (BD), and Jones Beach (JB) in spring, 1983.

starvation (Love 1980). When body lipids are as low as 1.5% they are believed to consist primarily of phospolipids which are a structural component of cell walls (Castledine and Buckley 1980; Love 1980). Further decline in lipid is unlikely since they are not available for use until cell destruction occurs.

There is some evidence that body lipids played a role in downstream survival of the fish that we sampled. As part of a study on downstream migrants, McKenzie et al. (1982, 1983) estimated the survival of these marked fish to McNary Dam in 1982 and to Priest Rapids in 1983. Higher downstream survival was associated with higher percent body lipid at release (Table 1). The nature of this relationship is unknown as the lower lipid level at release may have been coincident with factors that affected survival (transportation, stress, disease). An alternative explanation may be that lower survival of certain groups reflected less available body lipids in excess of 1.5%. Since some release groups started the migration at lower percent body lipid, they may have exhausted lipids in excess of 1.5% earlier in the migration and subsequently experienced lower survival.

The 65% decline in percent body lipid in seaward migrating chinook salmon smolts resulted in a significant decrease in caloric density (Appendix 1C). The decline in caloric density was expected to reduce mean total caloric value as seaward migration progressed. However, in 1982 and 1983 there were concurrent increases ($P<0.05$) in body weight and length as downstream movement progressed (Appendix 1D and 1E). The increase in body weight was sufficient to offset the

Table 1. Mean percent body lipid at release of spring chinook salmon of Leavenworth NFH origin released into the mouth of the Methow River. Percent survival to McNary Dam in 1982 and to Priest Rapids Dam in 1983 was calculated by Mckenzie et al. (1982, 1983).

	Brand	Percent lipid x	Percent lipid SD	Percent survival
1982				
	IL-1	4.4	1.2	40.2
	IL-3.	4.8	0.9	38.6
	IZ-1	4.6	1.8	39.3
	IZ-3	3.0	1.5	31.8
1983				
	IL-1	5.1	0.4	53.8
	IZ-2	4.3	1.6	40.5

decline in caloric density, precluding any changes in mean total caloric value.

Rapid growth in length and weight are characteristic of the parr-smolt transformation (Hoar 1976). Withey and Saunders (1973) suggested that lipid catabolism provides the energy for increased protein synthesis during smoltification, an observation consistent with our results. Total caloric values of fish serially sampled during the migration did not change because the decline in body lipid coincided with increases in the weight of the protein constituent although percentage remained the same. Protein synthesis resulted in increased length and weight but no real growth in total caloric value of smolts.

Changes that are characteristic of smoltification occurred with greater magnitude among fish released into the river than those held at the hatchery or laboratory (Fig. 5). Only two weeks after release ATPase activities had increased approximately four fold. ATPase activities of fish held in captivity later peaked at approximately 20 µmol Pi/mg protein/hour while levels in released fish reached activity levels greater than 40 µmol Pi/mg protein/hour. Lipids responded in an inverse manner. The percent body lipid of fish held in captivity declined only about 15%, but lipids of fish that were released declined 65% (Fig. 5). The high ATPase activity and precipitous loss of lipids that occurred in fish after release was indicative of a post-release period with high metabolic demand and intensified smoltification compared to fish retain in the hatchery or laboratory.

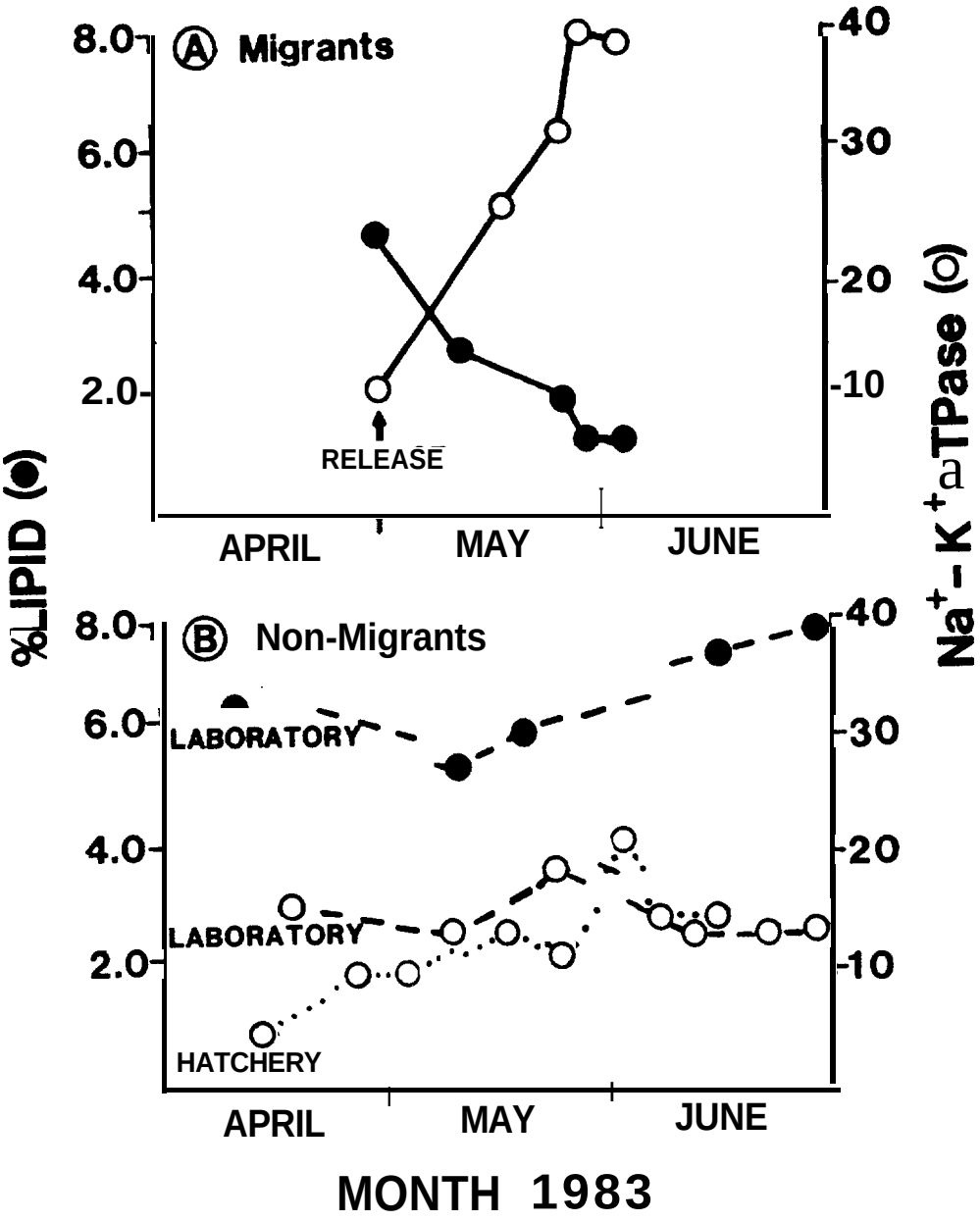

Figure 5. Percent body lipid and Na^+-K^+ ATPase of spring chinook salmon migrants compared to non-migrants. The arrow (↑) indicates day of release.

Seaward migration of chinook salmon smolts released from hatcheries can be conceived as occurring in two phases (Fig. 6). The first phase of the migration includes the time period when percent body lipids undergo a decline to about 1.5% and ATPase activities increase from pre-release levels to stable values between 30 and 50 µmol Pi/mg protein/hour. The second phase includes the remainder of the seaward migration that takes place in fresh water. The complexities of the smoltification process occurring during the first phase make it impractical to model. However, the second phase is of relevance to this project since it is this period when migration would be most affected by impoundments or change in flows. The bioenergetics model will, therefore simulate the energetics of smolts during the second phase.

In summary, a 65% decline in percent body lipids, a decline in body caloric density, and a four fold increase in ATPase activity of smolts occurred shortly after release from a hatchery. These changes were indicative of a post-release intensification of smoltification. The migration appeared to be a prerequisite to the intensified smoltification since fish held in the laboratory or hatchery did not respond similarly. Characteristics of smoltification were used to identify two phases during the seaward migration of spring chinook salmon smolts. Although substantial changes occurred in body constituents during phase I, there were no significant changes in total caloric value of smolts.

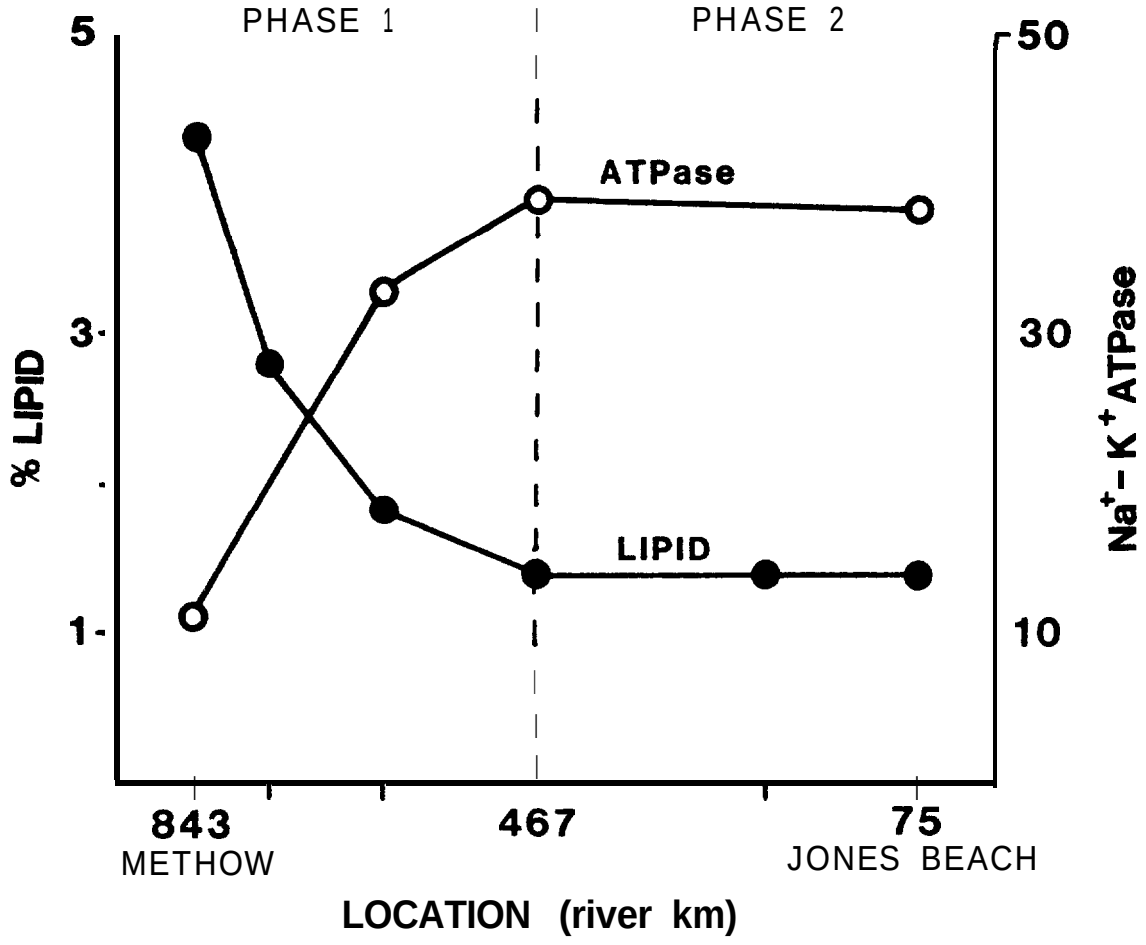

Figure 6. Phases of seaward migration and changes in percent body lipid and Na+-K+ ATPase activity of spring chinook salmon smolts.

FOOD CONSUMPTION - C

Objective 2: Estimate daily caloric intake (Cal/fish/day) of spring chinook salmon smolts during seaward migration.

Introduction

Daily food consumption of fish can be estimated using several indirect methods or by directly measuring the consumption rates. Indirect methods are usually based on energy requirements, growth rates, or an accepted relationship between food consumption, fish size, and water temperature. Since no information on food consumption by spring chinook salmon smolts in Columbia River reservoirs was available to verify indirect estimates, we selected a direct method described by Elliott and Persson (1978). This method uses gastric evacuation rates and mean weight of food in the stomachs of fish at selected time intervals during a 24-hour period to estimate daily food consumption (g food/fish/day). This approach has been validated by Elliott and Persson (1978) using laboratory experiments.

Quantitative estimates of food consumption should consider consumption by caloric value of food consumed as well as by weight. Daily caloric intake can be estimated from daily consumption if the caloric density of the food (cal/g dry weight) is known. It may be appropriate to assume a constant caloric density for food consumed if

the fish feed on one or a few types of prey items. However, for more opportunistic fishes consuming many different species of prey with different caloric densities, this assumption may not be valid and may bias estimates of the total caloric value of food consumed. Since, chinook salmon smolts feed on many different invertebrates we could not assume a constant caloric density without the possibility of biasing estimates of daily food consumption. Our approach was a stepwise procedure including determination of stomach content weights and caloric density over short time intervals, calculation of daily food consumption, and estimation of daily caloric intake.

Methods

Spring chinook salmon smolts were collected during seaward migration by midwater trawl from John Day and Wanapum reservoirs on the Columbia River. Collections in Wanapum Reservoir were made at Crescent Bar, river kilometer (Rkm) 707, on May 3-4, 1983 (Fig. 2). Collections in John Day Reservoir were made near Arlington, Oregon (Rkm 395) on May 19-20, 1982 and 1983 (Fig. 2). Approximately 20 fish were randomly selected from trawl catches at 4-hour intervals during a 24-hour period. Fish were immediately frozen on dry ice for later analysis.

In the laboratory, fish were thawed, weighed ($\pm$ 0.1 g), and measured to the nearest millimeter fork length. The stomach was excised and the contents were weighed ($\pm$ 0.1 mg). Dry weights of stomach contents were determined after drying for 24 hours at 65 C, and ash weights were determined after 3 hours in a muffle furnace at 600 C.

Caloric densities (cal/g dry weight) of stomach contents were estimated for each 4-hour interval. Stomach contents of five smolts were randomly selected for each time period and caloric density determined using a Phillipson microbomb calorimeter (Phillipson 1964). If a stomach contained less than 20 mg of food dry weight, a caloric estimate was not possible, and another fish was randomly selected. The dried prey items were homogenized, compressed into a pill weighing from 10-145 mg ($\pm$ 0.1 mg), and burned in the calorimeter. Data were corrected for acid production from the pill and heat gain from the fuse wire (Paine 1971). Caloric density of stomach contents were reported as cal/g dry weight.

Statistical analysis was used to determine if weight of stomach contents varied significantly during the 24 hour sampling period. Equality of variances within time periods was tested using the F_{max}-test (Sokal and Rolf 1981), and if neccessary, a loge transformation was used to remove heterogeneity of variances. When significant differences among the mean dry weights of stomach contents were detected by analysis of variance, the Neuman-Keuls multiple range test was used for comparisons among means to identify the significant differences.

Daily food consumption by juvenile chinook salmon was estimated by summing the estimated dry weight of food consumed in each of six four hour time periods (Elliott and Persson (1978)):

$$C_t = \frac{(S_t - S_0 e^{-R_t}) R_t}{1 - e^{-R_t}}$$

where: C_t = food consumed in 4-hour period;

S_0 = stomach content at beginning of time period;

S_t = stomach content at end of time period; and

R_t = exponential rate of gastric evacuation.

The rate of gastric evacuation was estimated by assuming that the time period with the largest proportional decline in stomach contents $[(S_0 - S_t)/S_0]$ occurred during a period of zero food consumption. Using this assumption and letting Ct = 0, the equation can be solved for Rt.

Daily caloric intake was estimated by multiplying the weight of food consumed during each time interval by the caloric density and summing those values for the six periods. The daily ration was expressed as a percent of body weight of the fish and was computed by dividing the estimated wet weight of food consumed per day by the mean wet weight of fish and multiplying by 100 (g food/day ÷ mean fish weight (g) x 100).

Results and Discussion

The mean dry weight of stomach contents of fish from Arlington, 1982, and Crecent Bar, 1983, were significantly higher ($P<0.05$) during late afternoon and evening time periods (Fig. 7; Appendix 2A and 2B). Although stomach analysis of fish from Arlington in 1983 showed a similar pattern, the differences were not significant (Appendix 2C).

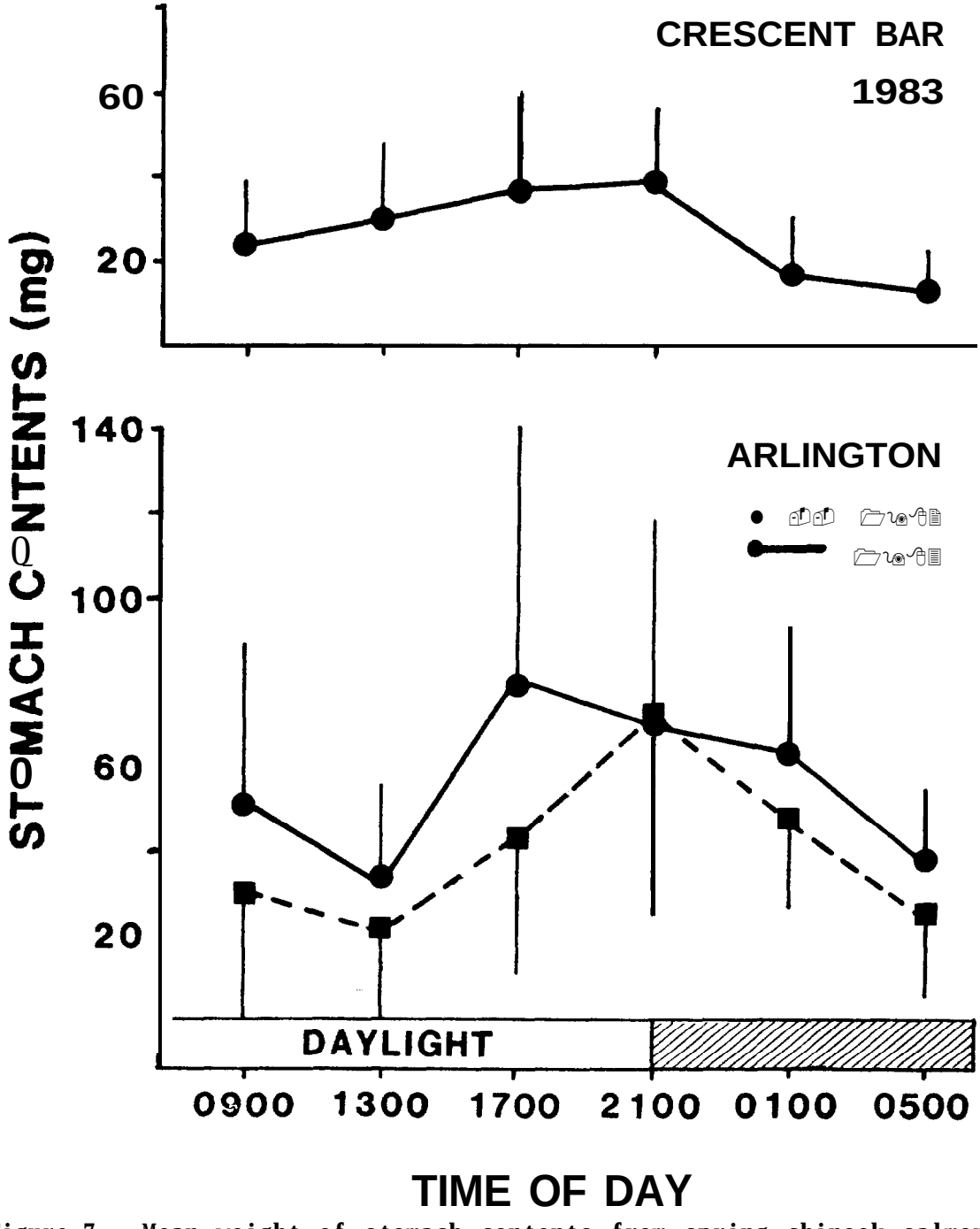

Figure 7. Mean weight of stomach contents from spring chinook salmon smolts at 4-hour intervals over a 24-hour period. Fish were collected at Arlington (Rkm 395) in 1982 and at Arlington and Cresent Bar (Rkm 707) in 1983. Vertical lines are standard deviations around the means.

The pattern of higher mean stomach contents in late afternoon indicated feeding occurred primarily during the day and evening hours.

Stomach contents of fish collected at Crescent Bar weighed less than stomach contents of fish at Arlington in both years sampled (Fig. 7). Fish sampled at Crescent Bar may have been recently released hatchery fish, and therefore were not adapted to feeding on natural prey items. Fork length of fish collected at Crescent Bar ranged from 120-140 mm, a larger size than expected for up-river wild spring chinook salmon smolts in April or May (80-110 mm) (French and Wahle 1959). Fish taken at Arlington (1982 and 1983) included fish of a wider range in size (90-150 mm) suggesting that both hatchery and wild or subyearling and yearling fish were caught.

Daily food consumption and ration were estimated for smolts from Arlington and Crescent Bar (Table 2). Daily rations (% of body weight) in Table 1 were standardized for a 19 g fish at 10 C. The estimated daily ration for 1983 at Arlington was 3.4% of body weight while the estimated daily ration for 1982 was 2.9%. The daily ration of fish collected at Cresent Bar (2.2%) was lower than either estimate for fish collected at Arlington.

Caloric densities of stomach contents collected at Arlington in 1982 were compared to data collected in 1983. Time of day was the only factor that contributed a significant amount to the caloric variation ($P<0.05$) (Appendix 2Da). Significant differences between time periods existed only between the highest and the two lowest mean caloric values (Appendix Table 2Db). This implies that differences in the caloric density between 1982 and 1983 were less important than diel differences

Table 2. Daily food consumption and daily ration of chinook salmon smolts collected from the Columbia River in 1982 and 1983.

Location	Evacuation rate, R	Daily food consumption (mg food dry wt/ fish)	Mean percent water	Daily food consumption (mg food wet wt/ fish)	Mean fish weight (g)	Daily ration adjusted to 19 g fish at 10 C
Cresent Bar 1983	0.134	78.6	81.1	415.6	22.8	2.2
Arlington 1983	0.136	183.9	79.3	888.4	18.4	3.4
Arlington 1982	0.166	158.2	76.6	676.1	16.0	2.9

in caloric density due to changes in species composition of prey items. Corophium sp. comprised a relatively high percentage of the stomach contents (4.4% and 9.8%) during time periods when caloric density was low (Fig. 8).

Daily caloric intake of smolts sampled at Arlington in 1982 was estimated using a grand mean for all time periods, both years, (5486 cal/g). Caloric densities in time periods which reflected low (5335 cal/g) and high (5562 cal/g) Corophium content in the stomachs were also used to calculate daily caloric intake at Arlington (Fig. 8). Applying the grand mean for caloric density to the estimates of food consumed yielded 869 Cal/fish/day for fish collected at Arlington in 1982, while applying the caloric densities reflecting Corophium yielded 868 Cal/fish/day. The grand mean for caloric density was therefore assumed adequate for estimating daily caloric intake. Caloric intake for fish sampled in 1983 at Arlington was subsequently estimated to be 1004 Cal/fish/day.

To determine if the grand mean for caloric density from Arlington was comparable to the density from Crescent Bar, caloric analyses were completed on stomach contents of fish sampled during the 1700-2100 hour time period. Since maximum food consumption took place in this time period at both locations, it was assumed that caloric values of stomach contents from this time period accurately reflected the actual caloric values of prey items consumed. A one-way analysis of variance for caloric density values of stomach contents from Crescent Bar (mean 5347 cal/g) and Arlington (1982, 5582 cal/g and 1983, 5513 cal/g) collected at 1700-2100 hours revealed no significant differences ($P>0.05$;

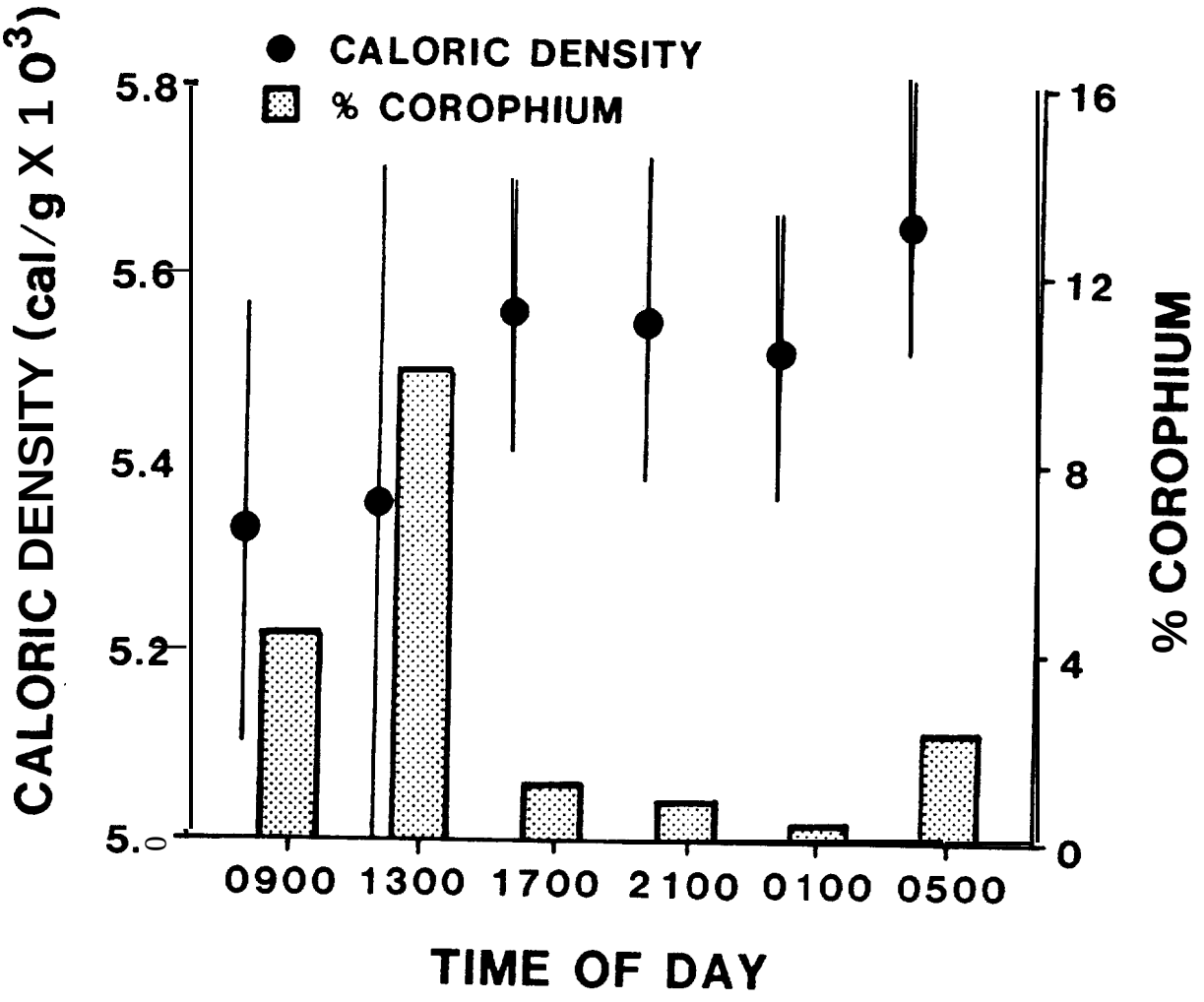

Figure 8. Caloric density of stomach contents of spring chinook salmon smolts with associated percent composition of Corophium. Fish were collected at 4-hour intervals over a 24-hour period. Vertical lines are standard deviations around the means.

Appendix Table 2E). A grand mean of 5486 cal/g calculated from Arlington data was therefore applied to the caloric density of food consumed by fish to estimate daily caloric intake (427 Cal/fish/day) at Cresent Bar.

SWIMMING ACTIVITY AND FOOD CONSUMPTION

Objective 3: Determine effect of swimming activity (Ra) and food consumption (C) by smolts on growth (AB) and survival in seawater challenge.

Introduction

The amount of energy used for swimming activity by juvenile chinook salmon may change with the onset of smoltification and seaward migration. Non-migratory parr expend a relatively small amount of energy since they tend to be territorial and sedentary. However, juvenile salmonids tend to become less territorial and more active with the onset of smoltification (Hoar 1976; Grau et al. 1982). Smoltification is also accompanied by reduced swimming proficiency and lowered stamina (Smith 1982). These changes are probably adaptations for migration under free flowing conditions when higher water velocities displace migrating juvenile salmon downstream. In recent decades impoundments have lowered water velocities and slowed migration rates (Raymond 1979), necessitating longer periods of active swimming by smolts in reservoirs rather than drifting with the current.

In addition to the energy expended on swimming and smoltification, smolts of hatchery origin apparently adapt slowly to the natural food sources after release. The observed decline in lipids can be

attributed primarily to smoltification, as has been described in objective one, but lipids are also mobilized during periods of starvation. Findings of this study (objective two) indicate that recently released yearling spring chinook salmon may have a relatively low daily ration. Ledgerwood (National Marine Fisheries Service, Hammond Bay Field Station, Hammond Bay, Oregon, pers. comm.) also observed low quantities of food in the stomachs of salmon smolts of hatchery origin entering the Columbia River estuary. These observations agree with those of other investigators who found that hatchery released fish have difficulty adjusting to a new feeding regime and environment, resulting in post release starvation (Hochachka 1961; Ersbak and Haase 1983; O'Grady 1983).

To determine the relative effect of prolonged swimming activity and starvation on the depletion of lipids, fish were subjected to known levels of the two factors in laboratory experiments. Seawater challenge was used to test the smolts adaptation to seawater after undergoing various levels of swimming activity and feeding ration. The relationship between survival in seawater and survival and growth after ocean entry has not been adequately described, but seawater challenge is a widely accepted test of hypo-osmoregulatory ability, a necessity for survival in the ocean.

Methods

Two experiments, one with an advanced photoperiod and the second with a natural photoperiod, were conducted with similar treatments.

In the first experiment, juvenile spring chinook salmon from Little White Salmon NFH were subjected to a photoperiod advanced approximately five weeks earlier than normal. The advanced photoperiod accelerated the onset of smoltification, allowing two experiments to be conducted in the same year. The first experiment lasted 34 days, starting April 22 and ending May 26. Water temperatures increased during the experiment from a daily mean of 10.1 C to 11.8 C with ± 1 C diel fluctuations. In the second experiment juvenile spring chinook salmon from Carson NFH, the same stock as sampled in objective one, were held under natural photoperiod conditions. The experiment began May 27 and ended June 27, lasting 31 days, during which the daily mean temperature rose from 10.1 C to 11.7 C with ± 0.7 C diel fluctuations.

Groups of fish in each experiment were subjected to two treatments, swimming activity and feeding regime. Each treatment had two levels resulting in a block design for each experiment as follows:

	Feeding Regime Treatment	
Swimming Treatment	Fed	Starve
Active	X	X
Inactive	X	X

Fish were subjected to forced swimming (active) or volitional swimming (inactive). Active fish were forced to swim at approximately 1.5 body lengths/second for 16 hours/day during the experimental period. The swimming speed of 1.5 body lengths/second approximated the speed at

which Besner and Smith (1983) considered the most energetically efficient, and therefore a likely cruising speed for migrating smolts. Theoretical evidence presented by Weihs (1973) also indicated 1.5 body length/second was an optimum swimming speed for fish that maximized the distance traveled and minimized energy expended. Inactive fish were held in circular tanks 1.5 m in diameter with minimal water current.

Food rations were calculated using weekly mean fish weight and water temperature on the day prior to feeding. Fish subjected to the fed treatment level were fed Oregon Moist Pellet formula 2 (OMP) once each day during the experiments at the rate of 3.0% of their wet body weight per day. Fish in the starved treatment level were not fed during the experiments.

Gill Na^+-K^+ ATPase activity was used as an index of smoltification. Ten fish were collected from each treatment group at the start of the experiment, at about two weeks, and again at the end of the experiment. Na^+-K^+ ATPase activity was determined by the method of Zaugg (1982).

Fish collected for analysis of body constituents were frozen and analyzed later for lipids, water, ash, and protein. Estimation of mean caloric value of smolts were performed as described in the methods of objective one.

The mean caloric value and percent lipid of smolt bodies at the end of each experiment were tested with a two-factor analysis of variance. Percent lipid data was transformed using an arcsine transformation prior to analysis.

Precocious males were present in both stocks of fish used in the experiments. Their presence was not considered important until it was

noticed that they did not survive the seawater challenge in the advanced photoperiod experiment. Subsequently, sex was determined for fish sampled during the natural photoperiod experiment. As a result, precocious males were excluded from the data analysis of the natural photoperiod regime, while the advanced photoperiod data included precocious males.

At the end of the experiments, fish were moved directly from fresh water to synthetic seawater at 30°/°° for a 96-hour seawater challenge. Two replicates of 15 fish each were randomly sampled from each treatment level and subjected to seawater challenge. Initial density was approximately 5.5 g fish/l of water. Challenges were conducted in 120 litre plastic containers and the water was filtered with a diatomaceous earth filter daily, oxygenated continuously, and changed every 48 hours. Dead fish were removed every 24 hours.

The data on survival of smolts in seawater was analyzed using a three-dimensional contingency table for each experiment (Zar 1984). Each dimension or factor of a contingency table had two levels: swimming activity (active, inactive), feeding regime (fed, starved), and survival in seawater (survivors, mortalities).

Results and Discussion

Fish used in the experiments were best described as psuedosmolts since they had not acquired the post-release characteristics of smolts described in results of objective two. In addition, the experimental treatments were initiated after ATPase activity had increased in order

to coincide with the date which low river flows and impoundments were likely to extend the migration time. Therefore, at the time of the seawater challenge the fish were considered post-smolts, although the term smolt is used throughout the results.

Swimming activity had significant effect on total caloric value or percent body lipid of smolts ($P>0.05$, Appendix 3A, 3B). Results were similar for advanced and natural photoperiod experiments.

Starvation caused a significant loss of body lipid in smolts in both experiments ($P<0.05$, Appendix 3A, 3B). Percent body lipid declined approximately 25-50% in the starved fish and increased slightly in fed groups (Fig. 9). Percent lipid in the fish starved and forced to swim at 1.5 body lengths/second declined 23-30% to 2.9% of body weight. In comparison, migrating smolts described in results of objective two exhibited a 65% decline to 1.4% lipid in about one-half the time period. Migrant and experimental smolts experienced similar water temperatures. The difference may reflect the degree of smoltification of the two groups, since smolts collected from the river had characteristics of more developed smolts.

Total caloric values of fish in the fed groups were significantly higher than fish in starved groups (Appendix 3A, 3B). Mean caloric values of fish in fed groups approximately doubled during the experiments, and changed little in starved smolts (Fig. 10). The lack of total caloric change in starved fish suggests these fish were very efficient at conserving energy reserves. This result supports the unchanging caloric values observed in smolts during migration despite relatively low food rations and loss of body lipids (objective two).

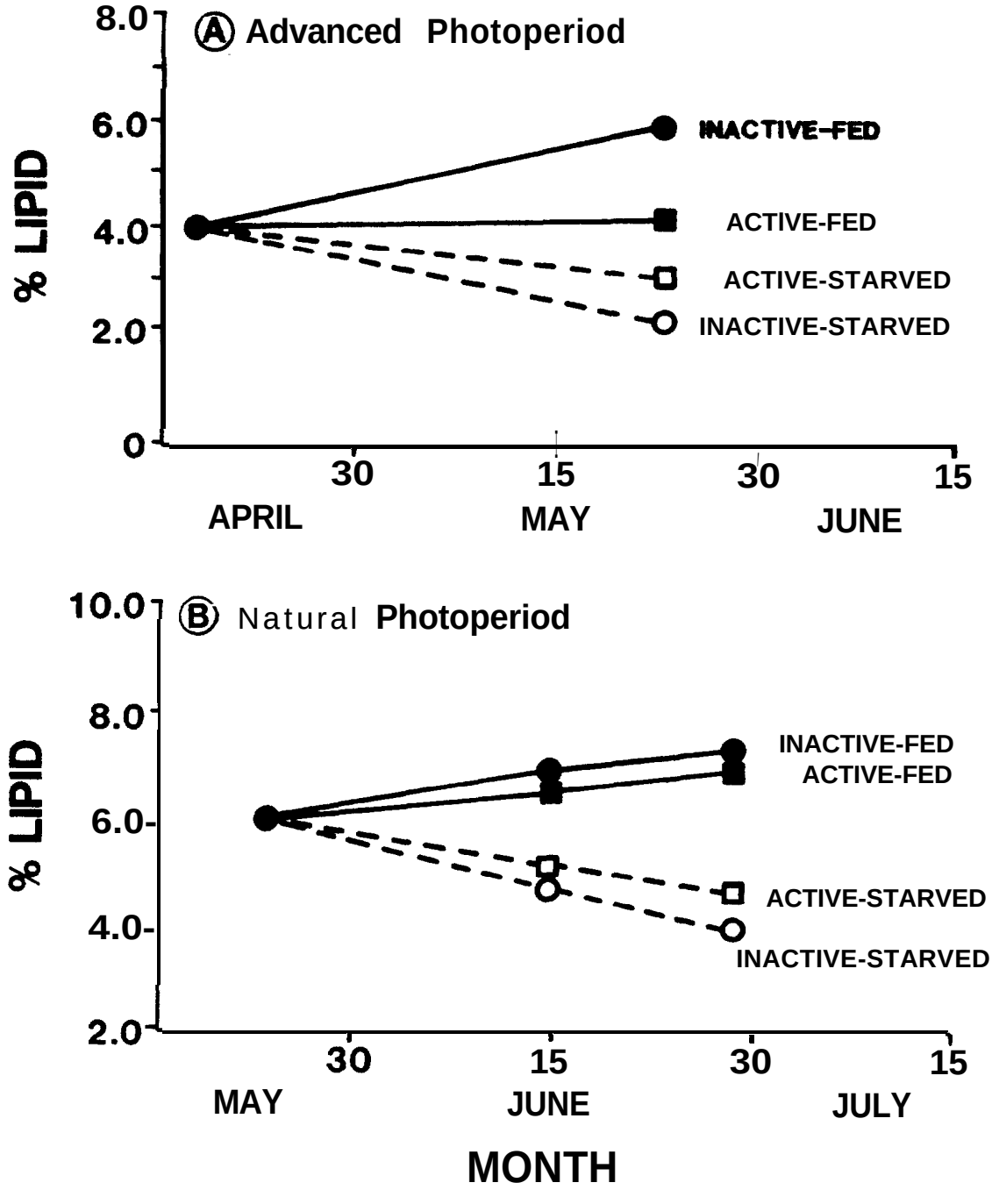

Figure 9. Mean percent body lipid (wet weight) of spring chinook salmon subjected to swimming activity (active and inactive) and feeding regime (fed and starved) treatments for 35 days in each of two experiments in 1983.

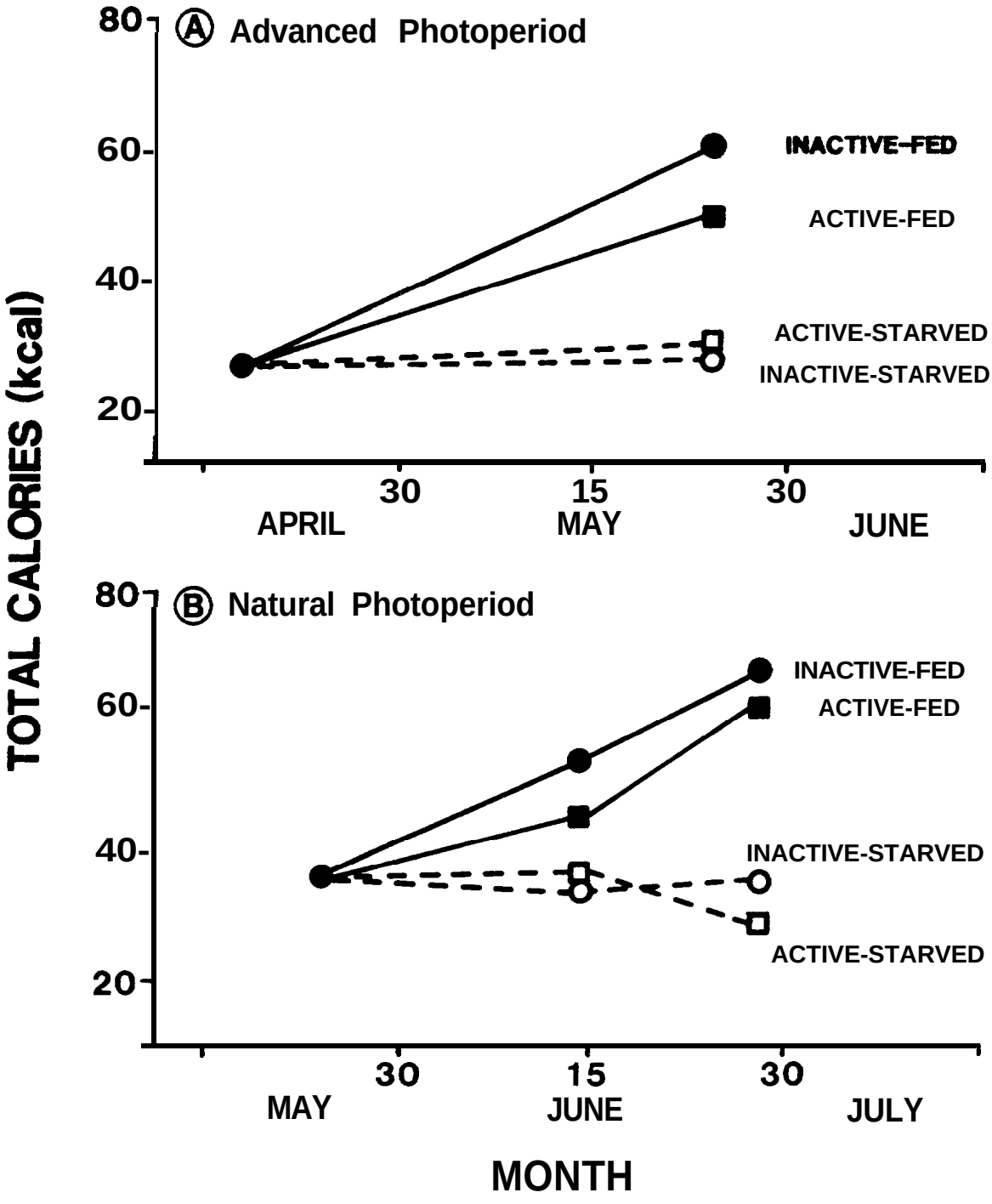

Figure 10. Mean total caloric value of spring chinook salmon subjected to swimming activity (active and inactive) and feeding regime (fed and starved) treatments for 35 days in each of two experiments in 1983.

As with body lipids and caloric values, survival in the seawater challenge tests was influenced primarily by feeding regime (starved and fed) rather than swimming activity in both experiments (Appendix 3C). Combining both levels of swimming, survival of fed fish was 55% higher than survival of starved fish (Fig. 11).

Survival of smolts in seawater was independent of swimming activity in the natural photoperiod experiment ($P>0.05$), but survival was significantly reduced by active swimming in the advanced photoperiod experiment ($P<0.05$, Appendix 3C). Further analysis revealed that the significant difference was due to differences in survival of active and inactive fish within the starved treatment level. Starved and active swimming fish had 27% survival in seawater while starved inactive fish had 67% survival (Fig. 11).

The starved-active fish had significantly lower ATPase activity than other groups as early as two weeks after the start of the treatments ($P<0.05$). Reductions in ATPase activity may have been more pronounced in the advanced photoperiod than natural photoperiod fish because ATPase activities were higher and the experiment more nearly coincided the time of natural peak smoltification of spring chinook salmon. High ATPase levels and low swimming stamina are characteristic of smoltification. Therefore, forced swimming may have had a more deleterious effect on survival among the more completely smolted fish.

Weihs (1973) and Besner (1980) indicated a swimming speed of 1.5 body lengths/second was optimum. Smith (1982) noted that swimming proficiency and fatigue levels of smolts was so low that sustained swimming was not possible at velocities greater than 1.5 body

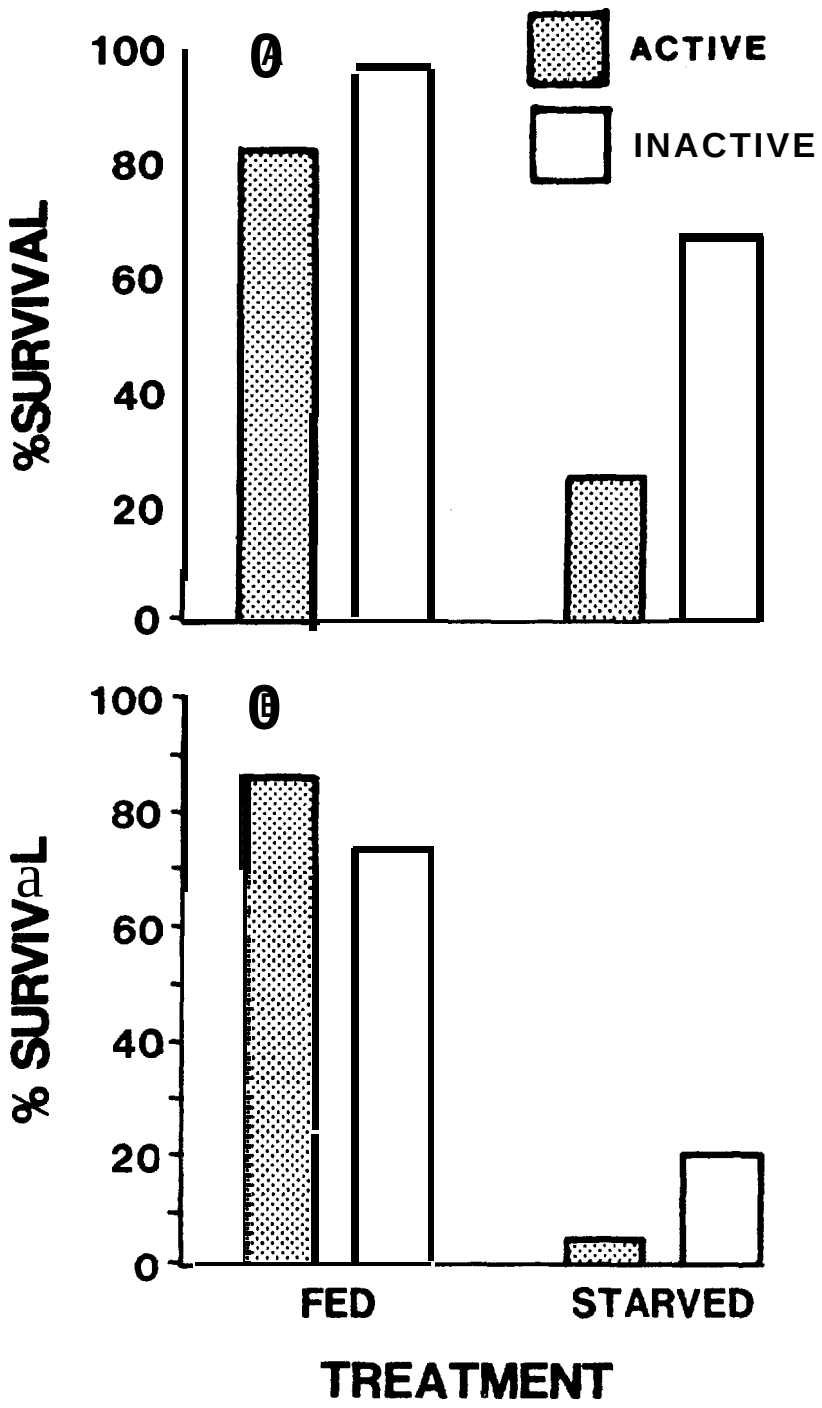

Figure 11. Percent survival of spring chinook salmon in a 96-hour seawater challenge after being subjected to swimming activity (active and inactive) and feeding regime (fed and starved) treatments in advanced (A) and natural photoperiod (B) experiments.

lengths/second (Smith 1982). Our results indicated swimming activity at 1.5 body lengths/second did not reduce survival of fish in seawater when sufficient food was available. Therefore, a maximum swimming speed of about 1.5 body lengths/second during periods of active migration will be used in the energetics model.

In summary, starvation, unlike swimming activity, exerted a strong influence on the energy reserves of smolts, as well as on survival in seawater challenge tests. If a similar situation exists for actively migrating smolts on a limited or zero food ration, survival may be reduced during seawater entry. Survival of fish in the active and inactive swimming groups was dependent on feeding regime, as demonstrated by chi-square contingency table analysis (Appendix 3C). Potential errors in assumptions about food consumption in model simulations of smolt energetics will be more serious than potential errors in assumptions on swimming activity.

DIETARY LIPID AND SURVIVAL

Objective 4: Determine effects of starvation and lipid loss on survival of smolts in seawater.

Introduction

The precipitous decline of body lipids observed in hatchery fish after release may be attributed to low food consumption (Hochachka 1961; O'Grady 1983), as well as smoltification (Sheridan et al. 1983). The loss of lipids in migrating smolts is of concern because of the implicated role of lipids in seawater survival. Wagner (1974) observed that steelhead trout with higher lipid reserves had a greater ability to adapt to seawater. Burrows (1969) obtained an 88% greater adult return in fall chinook salmon released at a body lipid level of 7.9% compared to fish released at 4.1%. Similarly, Peterson (1973) fed yearling Atlantic salmon (*Salmo salar*) a diet containing 16% marine fish lipid for a month before release and observed a near record adult return compared to fish fed the standard hatchery diet containing 6% marine fish lipid.

Marine fish lipids characteristically contain a high percent of $\omega 3^a$ fatty acids (Ackman 1967), which enhance growth and survival in seawater (La11 and Bishop, 1976; Takeuchi and Watanabe, 1982).

[a] A shorthand designation for fatty acids where ω number identifies the position of the first double bond counting from the methyl end.

Of the ω3 fatty acids, 22:5 and 22:6 or their precurser, 18:3, have been classified as essential to the diet for proper health of artificially propagated salmonids (Cowey and Seargent 1977: Watanabe 1982).

Laboratory experiments were designed to determine if additional dietary lipids high in polyunsaturated fatty acids (ω3) could compensate for subsequent starvation and improve short-term survival after seawater entry.

Methods

Yearling spring chinook salmon obtained from Carson NFH were divided into two equal groups and fed diets differing in lipid content. One diet was Oregon Moist Pellet II (OMP) which contained approximately 12% lipid by wet weight, and the other diet was a high lipid diet (HL-OMP) with tuna oil added to OMP for 19% lipid content. Tuna oil was selected because marine fish oils have a high percentage of ω3 fatty acids (Ackman 1967) and it is an ingredient of OMP-2. Lipid content of food was analyzed by the Abernathy Salmon Development Center, U.S. Fish and Wildlife Service.

The fish were fed at a rate of 3% of their wet body weight per day. The two groups of fish were fed their respective diets from April 1 - May 18 as water temperatures rose from 5 to 8 C.

Starting on May 18 fish were acclimated to heated river water while feeding continued. During the experiment, which began May 23, daily mean water temperatures rose from 10.1 C to 11.7 C with natural

diel fluctuations of about ± 0.7 C. On May 23 the two fed groups were divided and one group from each diet starved.

Feeding regime	Diet (% Lipid) OMP (12%)	HL-OMP (19%)
Fed	X	X
Starved	X	X

At the end of 5 weeks (May 23 - June 28) 30 fish from each of the four treatment groups were subjected to a 96-hour seawater challenge at 30°/oo. Density of fish and procedures of the seawater challenge were the same as described in methods for objective three.

ATPase activity was used as an indicator of smoltification, with gill samples obtained at two-week intervals throughout the experiment. Na^+-K^+ ATPase activity was determined by the method of Zaugg (1982).

Ten fish were collected for body lipid determination from each group at the start of the experiment, after two weeks, and the day before the seawater challenge. Body lipids were extracted by the same method as described earlier (objective 2).

Three fish from each of the four treatment groups were homogenized and pooled for fatty acid analysis. The separation of neutral and polar lipid fractions and subsequent fatty acid identification techniques used are described in detail by Mugrditchian et al. (1981).

Results and Discussion

In an attempt to conduct experiments at a time corresponding to when the seaward migration of smolts would be seasonally delayed in the river during a low flow year, we held fish in the laboratory until late May before starting the experiments. Implicit in the use of fish from the hatchery was the assumption that the fish were smolts and that they would be representative of smolts that experienced prolonged migration in late May and June of low-flow years. The results of Objective 2 have since indicated that these assumptions are questionable. The physiological response of laboratory fish during smoltification is suppressed compared to river migratory smolts, and we are inclined to use the term pseudosmolt to describe fish held in the laboratory.

The two pretreatment diets were established to create two groups with different body lipid levels prior to beginning the experiment. However, 50 days the pretreatment feeding failed to establish a significant difference in lipid levels between groups ($P>0.05$). The percent body lipid of fish fed OMP was 6.1% compared to 6.7% for fish receiving HL-OMP.

Sampling at the conclusion of the experiment revealed that feeding and starvation had a significant effect on percent body lipid of fish ($P<0.05$, Appendix 4A). Starvation reduced percent body lipid about equally within groups, causing a 33 and a 36% decline among fish fed pretreatment diets of OMP and HL-OMP, respectively. Overall, the decline in body lipids during starvation of about 35% was considerably less than the 65% decline observed in hatchery released smolts

collected at downstream locations on the Columbia River (objective 2). Level of dietary lipid did not significantly affect the percent body lipid in fish of fed or starved treatments ($P>0.05$, Appendix 4A).

Body weight after treatments was significantly different between the fed and starved groups ($P<0.05$, Appendix 4B). The mean weight of fed fish was 42 g and the mean weight of starved fish was 24 g. Level of dietary lipid did not significantly affect weight of fish at the end of the experiment. The mean weight of precocious males from all groups was 41.5 g compared to 33 g for other males.

ATPase activity had declined to what is typically considered a post-smolt level at the time of seawater challenge (Fig. 12). Of notable interest was the higher peak in ATPase activity attained by the fish fed HL-OMP prior to the experiment. Mean activity was 19.6 $\pm$ 3.8 and 14.2 $\pm$ 4.0 µmol Pi/mg protein/hour among fish fed HL-OMP and fish fed OMP, respectively. Fish receiving HL-OMP were also about 4.5 g larger than other fish. However, the differences in ATPase activity were not attributed to size differences since similar differences in ATPase activity were observed among other fish fed high lipid diets.

ATPase activity levels of the precocious males were suppressed throughout the period of smoltification (Fig. 12) and, not surprisingly, survival of the precocious males in the seawater challenge was poor. When all treatments were combined (some not reported here) only 2 of 29 (7%) of precocious males survived compared to 77 of 110 (70%) for the non-precocious males. Hence we excluded precocious males from the survival analysis.

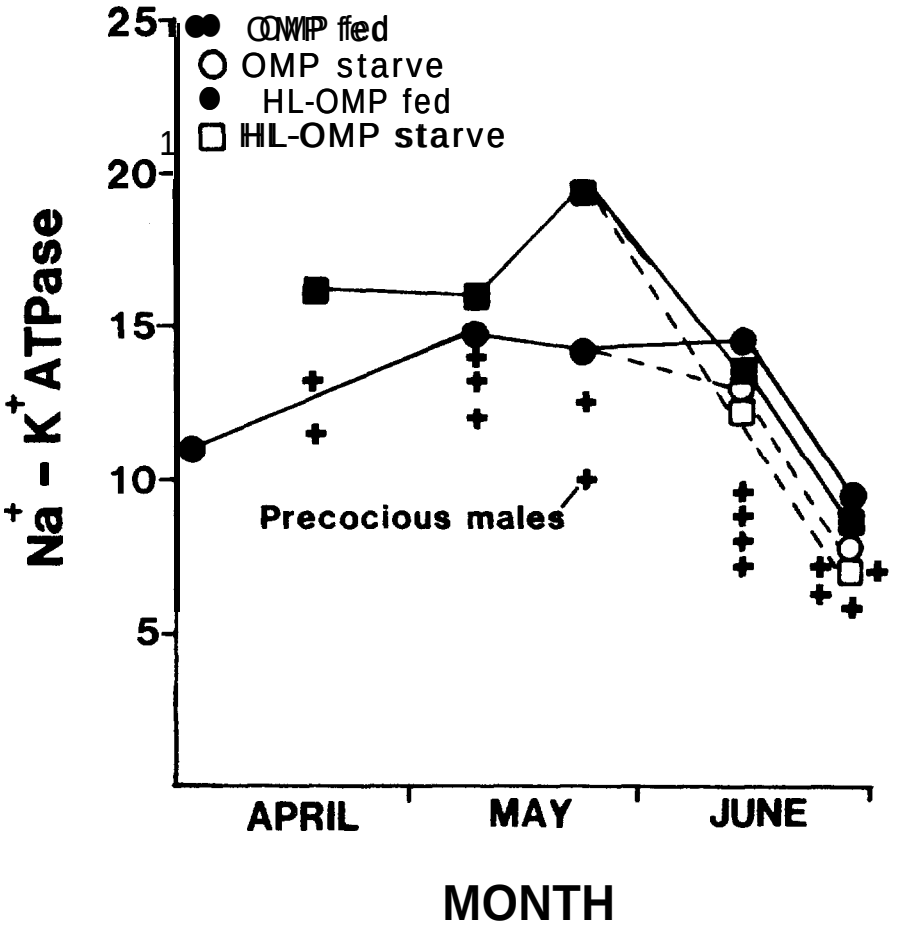

Figure 12. Mean Na^+-K^+ ATPase activity of yearling spring chinook salmon fed OMP and high lipid OMP.

Chi-square analysis indicated that survival in the seawater challenge test was influenced by feeding regime and pretreatment dietary lipid (Appendix 4C). Dietary lipid level was not, however, as important a determinant as feeding regime, again confirming the results presented in objective three. Survival in seawater was apparently associated with the percent body lipid at the beginning of the seawater challenge (Fig. 13).

There is evidence for the positive relation between survival in seawater and body size during and outside the period of smoltification (Parry 1966; Conte et al. 1966; Clarke 1982). There was no association in our data between body size and survival, unless the two fed groups were combined and compared to the two combined starved groups. Further, the precocious males were the largest of all the individuals in the experimental groups yet had nearly 100% mortality. Thus it appears that the physiological condition of the fish, and not body size in itself, was the factor determining survival.

Fatty acid profiles of the four groups of fish and precocious males are shown in Table 3. The ω3 fatty acids, implicated as beneficial for survival and growth in seawater, include the 18:3, 20:5, 22:5, and 22:6 fatty acids shown in Table 3. Starvation caused a reduction in the percent of the neutral ω3 essential fatty acids 22:6 and 20:5, while increasing the percent of 16:0, 18:1, and 20:1 neutral fatty acids.

Survival in seawater was directly proportional to the amount of ω3 fatty acids in the fish. Initial analysis indicated that ω3 fatty acids as a percent of the neutral lipid pool differed only slightly

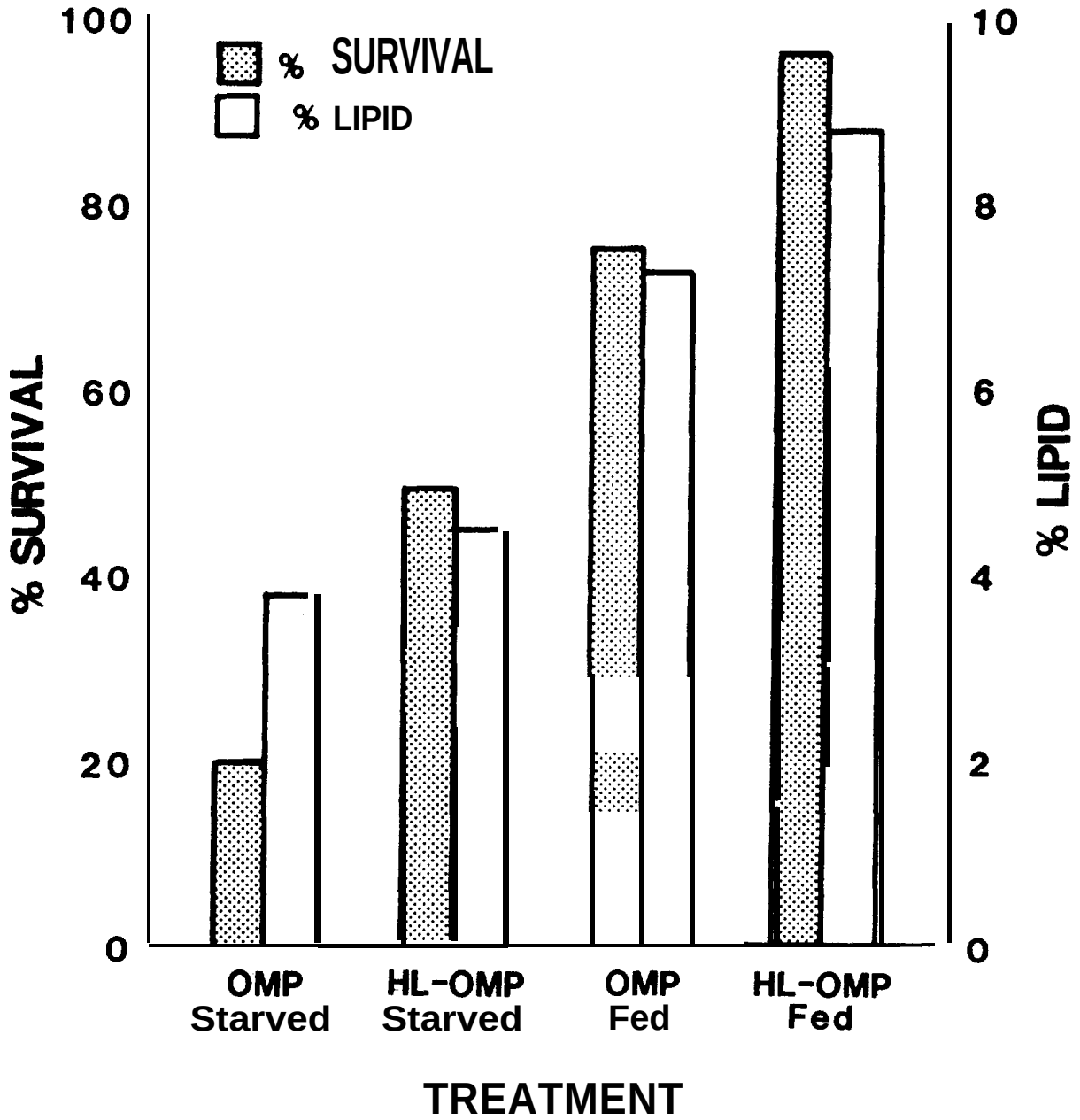

Figure 13. Percent survival of yearling spring chinook salmon in seawater challenge and associated body lipid content.

Table 3. Percent neutral and polar fatty acids in spring chinook salmon fed OMP (12% lipid) or high lipid OMP (19% lipid) for 50 days, and then starved or fed for an additional five weeks.

Fatty Acid	Treatment-Diet: Fed HL-OMP	Fed OMP	Starve HL-OMP	Starve OMP	Fed OMP[a]
NEUTRAL					
14:o	2.6	3.3	3.1	3.0	3.5
16:0	12.1	12.9	14.3	16.2	13.0
16:1	6.7	7.5	7.1	7.2	7.8
18:0	2.0	2.2	2.6	2.7	2.0
18:1	26.2	27.8	30.5	29.1	28:8
18:2	16.7	13.5	16.0	14.3	14.0
18:3	0.8	0.7	0.8	0.8	0.7
20:1	4.2	2.1	5.1	5.8	2.3
20:4	1.1	3.4	3.3	3.7	3.3
20:5	7.7	7.1	4.5	4.8	6.5
22:4	0.2	1.4	0.5	0.7	0.0
22:5	1.2	1.7	1.0	0.8	1.4
22:6	14.9	12.8	10.5	10.4	11.9
POLAR					
14:o			2.8	1.9	3.2
16:0			11.0	11.0	14.7
16:1			3.2	4.7	6.5
18:0			4.4	4.3	1.8
18:1			26.1	26.0	21.0
18:2			7.9	6.0	5.9
18:3			0.3	0.4	1.8
20:1			3.9	4.0	6.5
20:4			4.5	5.2	2.9
20:5			4.5	5.1	5.2
22:4			2.2	0.7	1.8
22:5			26.0	26.6	4.4
22:6					14.4

a Precocious males fed OMP, sampled just prior to the saltwater challenge.

among starved groups (16.8% - 16.5%), though survival ranged from 20-49%. Subsequent analysis revealed that the amount of ω3 fatty acids available on a percent body weight basis corresponded more closely with percent survival than percent ω3 in the neutral lipid pool (Table 4). Two essential fatty acids, 20:5 and 22:6, made up the majority of the ω3 pool, and our results indicate that they play a role in the seawater adaptation process.

Results from these experiments indicate that neutral fatty acids of the ω3 class may play an important role in the process of seawater adaptation. We are not certain, though, that these results are applicable to true migratory smolts which have a fully functional Na^+-K^+ ATPase active ion transport system. More likely, these results are applicable to fish that have not yet smolted fully, such as fish transferred directly from the hatchery to seawater. Supplementation of ω3 fatty acids to the diet may enhance short term survival in these situations.

Table 4. Neutral ω3 fatty acids as a percent of total neutral fatty acids and as a percent of body weight. The ω3 fatty acids pooled are 18:3, 20:5, 22:5, and 22:6.

	Treatment-Diet			
	Fed HL-OMP	Fed OMP	Starve HL-OMP	Starve OMP
% ω3 of neutral	24.6	22.3	16.8	16.5
total weight ω3 (mg)	827	564	146	109
% ω3 of total body weight	1.90	1.38	0.59	0.46
% survival	96	74	49	20

SUMMARY

1. Mean caloric value of spring chinook salmon smolts (B of the model) remained constant throughout smoltification and migration. This occurred because an increase in body weight during migration negated the precipitous decline in caloric density.

2. Smoltification in laboratory or hatchery held fish was suppressed and it appeared migration plays an integral part in smolt development.

3. A 65% decline in percent body lipids and a four fold increase in gill Na^+-K^+ ATPase activity after release indicated intensified post-release smolt development early in the seaward migration of smolts.

4. Seaward migration of hatchery reared spring chinook salmon was separated into two phases. Phase I was defined as the period of intensified smolt development; this phase of the migration will not be modeled. Phase II was defined as an anticipatory period in which fish maintain a fully functional smolt status until they reach the estuary. Phase II would be the period most affected by yearly changes in water flow and will be the period modeled.

5. Caloric intake by migrating smolts ranged from 427 Cal/fish/day in the Columbia River near Cresent Bar, Washington to 1004 Cal/fish/day near Arlington, Oregon.

6. Caloric density of prey items consumed by smolts did not differ between years, but was lower when Corophium was abundant in the diet.

7. Daily ration for migratory spring chinook salmon smolts ranged from 2.2-3.4% body weight for a 19 g fish at 10 C.

8. Forced swimming at 1.5 body lengths/second did not affect percent body lipid or mean caloric value of smolts. Forced swimming reduced subsequent survival in seawater only for smolts that had developed the highest Na^+-K^+ ATPase activity.

9. Starved smolts had lower percent body lipid, mean caloric value, and survival in seawater challange tests than fed smolts. We were unable, however, to duplicate in the laboratory the rate and magnitude of the body lipid decline observed in river migrants, despite forced activity and starvation.

10. Increased survival of smolts in seawater challenge tests was directly associated with an increased percentage of specific $\omega 3$ neutral fatty acids in the body. This observation was of interest

because the neutral lipid pool in migratory smolts was near exhaustion before one half of the migration was completed.

LITERATURE CITED

Ackman, R.G. 1967. Characteristics of the fatty acid composition and biochemistry of some freshwater oils and lipids in comparison with marine oils and lipids. Comparative Biochemistry and Physiology 22:907-922.

Baraduc, M.M. and M. Fontaine. 1955. Etude comparee du metabolisme respirtoire du jeune saumon sedentaire (parr) et migrateur (smolt). Societe De Biologie: 1327-1329.

Besner, M. 1980. Physical training: An affordable strategy to increase food conversion efficiency, stamina, growth and survival of coho salmon smolts (Oncorhynchus kisutch). Doctoral dissertation. University of Washington, Seattle, Washington, USA.

Besner, M. and L.S. Smith. 1983. Modification of swimming mode and stamina in two stocks of coho salmon (Oncorhynchus kisutch) by differing levels of long term continuous exercise. Canadian Journal of Fisheries and Aquatic Sciences 40:933-939.

Bligh, E.G. and W.J. Dyer. 1959. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37:911-917.

Brett, J.R. and T.D.D. Groves. 1979. Physiological Energetics. Pages 280-352 In W.S. Hoar, D.J. 'Randall, and J.R. Brett, editors. Fish Physiology, Volume,VIII. Bioenergetics and Growth. Academic Press, New York, New York, USA.

Burrows, R.E. 1969. The influence of fingerling quality on adult salmon survival. Transactions of the American Fisheries Society 98:777-784.

Castledine, A.J. and J.T. Buckley. 1980. Distribution and mobility of ω3 fatty acids in rainbow trout fed varying levels and types of dietary lipid. Journal of Nutrition 110:675-685.

Caulton, M.S. and E. Bursell. 1983. The relationship between changes in condition and body composition in young Tilapia rendalli Boulenger. Journal of Fish Biology 11:143-150.

Clarke, C.W. 1982. Evaluation of the seawater challenge test as an index of marine survival. Aquaculture 28:177-183.

Congleton, J.L., **T.C. Bjornn,** B.H. Barton, B.D. Watson, J.I. Irving and R.R. Ringe. 1984. Effects of handling and crowding on the stress response and viability of chinook salmon parr and smolts. University of Idaho, Department of Fish and Wildlife Resources, Idaho Cooperative Fishery Research Unit. Completion Report Project 82-5 to Department of Energy, Bonneville Power Administration, Division of Fish and Wildlife, Portland, Oregon, USA.

Conte, F.P., H.H. Wagner, J.C. Fessler, and C. Gnose. 1966. Development of osmotic and ionic regulation in juvenile coho salmon (Oncorhynchus kisutch). Comparative Biochemistry and Physiology 18:1-15.

Cowey, C.B. and J.R. Seargent. 1977. Lipid nutrition in fish. Comparative Biochemistry and Physiology 57:269-273.

Elliott, J.M. and 1. Persson. 1978. The estimation of daily rates of food consumption for fish. Journal of Animal Ecology 47:977-991.

Ersbak, K. and B.L. Haase. 1983. Nutritional deprivation after stocking as a possible mechanism leading to mortality in stream-stocked brook trout. North American Journal of Fisheries Management 3:142-151.

Folmar, L.C. and W.W. Dickhoff. 1980. The parr-smolt transformation (smoltification) and seawater adaptation in salmonids: A review of selected literature. Aquaculture 21:1-37.

French, R.R. and R.J. Wahle. 1959. Biology of the blueback and chinook and steelhead in the Wenatchee River system. United States Fish and Wildlife Service, Special Scientific Report-Fisheries Number 304, Washington D.C., USA.

Gorbman, A., W.W. Dickhoff, J.L. Mighell, E.F. Prentice and F.W. Waknitz. 1982. Morphological indices of developmental progress in the parr-smolt coho salmon, Oncorhynchus kisutch. Aquaculture 28:1-19.

Grau, E.G., J.L. Specker, R.S. Nishioka and H.A. Bern. 1982. Factors determining the occurrence of the surge in thyroxine activity in salmon during smoltification. Aquaculture 28:49-57.

Hanson, S.W.F. and J. Olley. 1963. Application of the Bligh and Dyer method of lipid extraction to tissue homogenates. Biochemistry Journal 89:101-102.

Hoar, W.S. 1976. Smolt transformation: evolution, behavior, and physiology. Journal of the Fisheries Research Board of Canada 33:1234-1252.

Hochachka, P.W. 1961. Liver glycogen reserves of interacting resident and introduced populations. Journal of the Fisheries Research Board of Canada 18:125-135.

Lall, S.P. and F.J. Bishop. 1976. Studies on the requirements of rainbow trout (Salmo gairdneri) grown in sea water and fresh water. Pages 580-584 In T.V.R. Pillay and W.A. Dill, editors. Technical Conference on Aquaculture, FIR:AQ/Conference/76/E. 12. Kyoto, Japan. Food and Agriculture Organization of the United Nations, Rome, Italy.

Love, R.M. 1980. Chemical biology of fishes Volume 2: Advances 1968-1977, with a supplemental key to the chemical literature. Academic Press, New York, New York, USA.

Mckenzie, D., D. Weitkamp, T. Schadt, D. Carlile and D. Chapman. 1983. 1982 systems mortality study. Battelle, Pacific Northwest Laboratories, Richland, Washington. Prepared for Chelan County Public Utility District, Grant County Public Utility District, and Douglas County Public Utility District, Battelle Pacific Northwest Laboratories, Richland, Washington, USA.

Mckenzie, D., D. Carlile and D. Weitkamp. 1984. 1983 systems mortality study. Battelle, Pacific Northwest Laboratories, Richland, Washington. Prepared for Chelan County Public Utility District, Grant County Public Utility District, and Douglas County Public Utility District, Battelle Pacific Northwest Laboratories, Richland, Washington, USA.

Mugrditchian, D.S., R.W. Hardy and W.T. Iwaoka. 1981. Linseed oil and animal fat as alternative lipid sources in dry diets for chinook salmon (Oncorhynchus tshawytscha). Aquaculture 25:161-172.

O'Grady, M.F. 1983. Observations on the dietary habits of wild and stocked brown trout, Salmo trutta L., in Irish lakes. Journal of Fish Biology 22:593-601.

Paine, R.T. 1971. The measurement and application of the calorie to ecological problems. Annual Review of Ecology and Systematics 2:145-164.

Parry, G. 1966. Osmotic adaptation in fishes. Biological Review 41:392-444.

Peterson, H.H. 1973. Adult returns to date frorn hatchery-reared one-year old smolts. International Atlantic Salmon Foundation Special Publication 4:219-226.

Phillipson, J. 1964. A miniature bomb calorimeter for small biological samples. Oikos 15:130-139.

Raymond, H.L. 1979. Effects of dams and impoundments on migrations of juvenile chinook salmon and steelhead from the Snake River, 1966-1975. Transactions of the American Fisheries society 108:505-529.

Sheridan, M.A., W.V. Allen and T.H. Kerstetter. 1983. Seasonal variation in the lipid composition of the steelhead trout, Salmo gairdneri R., associated with parr-smolt transformation. Journal of Fish Biology 23:125-134.

Smith, L.S. 1982. Decreased swimming performance as a necessary component of the smolt migration in salmon in the Columbia River. Aquaculture 28:153-161.

Sokal, R.R. and F.J. Rolf. 1981. Biometry, 2nd edition. W.H. Freeman and Company, San Francisco, California, USA.

Takeuchi, T. and T. Watanabe. 1982. Effects of various polyunsaturated fatty acids on growth and fatty acid compositions of rainbow trout Salmo gairdneri, coho salmon Oncorhynchus kisutch, and chum salmon Oncorhynchus keta. Bulletin of the Japanese Society of Fisheries 12:1745-1752.

Wagner, H.H. 1974. Seawater adaptation independent of photoperiod in steelhead trout (Salmo gairdneri). Canadian Journal of Zoology 52:805-812.

Watanabe, T. 1982. Lipid nutrition in fish. Comparative Biochemistry and Physiology 73:3-16.

Webb, P.W. 1975. Hydrodynamics and energetics of fish propulsion. Fisheries Research Board of Canada, Bulletin 190.

Wedemeyer, G.A. R.L. Saunders and W.C. Clarke. 1980. Environmental factors affecting smoltification and early marine survival of anadromous salmonids. Marine Fish Review 42:1-14.

Weihs, D. 1973. Optimal cruising speed for migrating fish. Nature 245:48-50.

Winans, G.A. 1984. Multivariate rnorphometric variabililty in Pacific salmon. Canadian Journal of Fisheries and Aquatic Sciences 41:1150-1159.

Withey, K.G. and R.L. Saunders. 1973. Effect of a reciprocal photoperiod regime on standard rate of oxygen consumption of postsmolt Atlantic salmon Salmo salar. Journal of the Fisheries Research Board of Canada 30:1898-1900.

Zar, J.H. 1984. Biostatistical Analysis. Prentice-Hall, Englewood Cliffs, New Jersey, USA.

Zaugg, W.S. 1982. A simplified preparation for adenosine triphosphatase determination in gill tissue. Canadian Journal of Fisheries and Aquatic Sciences 39:215-217.

Appendix 1. Analysis of variance tables and Newman-Keuls tests for data used to estimate total caloric value (B) of spring chinook salmon smolts at selected locations during seaward migration in the Columbia River.

Appendix 1A.

a). Analysis of variance of total caloric values of the IZ-2 branded release groups of hatchery reared spring chinook salmon smolts sampled at six locations during seaward migration in the Columbia River, 1983.

Source	df	MS	F-ratio
Between stations	5	35.19	0.83 NS
Within stations	59	43.57	
Total	64		

b). Analysis of variance of total caloric values of four branded release groups of hatchery reared spring chinook salmon smolts, sampled at five locations during seaward migration in the Columbia River, 1982.

Source	df	MS	F-ratio
Station	4	101.63	1.40 NS
Release group	3	18.03	0.25 NS
Station x release	12	89.04	1.22 NS
Error	235	73.75	

Appendix 1B.

a). Analysis of variance for arcsine of percent total lipids of the IZ-2 branded release group of hatchery reared spring chinook salmon smolts sampled at six locations during seaward migration in the Columbia River, 1983. Asterisk (*) denotes P<0.05.

Source	df	MS	F-ratio
Between locations	5	38.03	25.613 *
Within locations	59	1.48	
Total	64		

b). Newman-Keuls test for mean percent lipid of chinook salmon smolts at selectted locations during the seaward migration of 1983. Underlined values are not significantly different (P>0.05).

	Location					
	Jones Beach	Bonneville Dam	McNary Dam	Priest Rapids Dam	Rocky Reach Dam	Leavenworth NFH
Mean percent lipid wet weight	1.4	1.4	1.4	1.8	2.8	4.3

Appendix 1C.

a). **Analysis of variance of caloric density values for IZ-2 branded release group of hatchery reared spring chinook salmon smolts, sampled at six locations during seaward migration in the Columbia River, 1983. Asterisk (*) denotes P<0.05.**

Source	df	MS	F-ratio
Between stations	5	0.132	22.4 *
Within stations	61	0.005	
Total	66		

b). **Newman-Keuls test for mean caloric density of chinook salmon smolts sampled at selected locations during the seaward migration of 1983. Underlined values are not significantly different (P<0.05).**

	Location					
	Jones Beach	Bonneville Dam	McNary Dam	Priest Rapids Dam	Rocky Reach Dam	Leavenworth NFH
Mean Kcal/g wet weight	1.02	1.02	1.03	1.05	1.16	1.31

Appendix 1D. Mean wet weight and sample size of spring chinook salmon smolts collected in 1982 and 1983. Fish were collected at Leavenworth NFH the day before their release into the mouth of the Methow River. All brands collected in 1982 (IL-l, IL-3, IZ-1, IZ-3) and 1983 (IL-l, IZ-2) were combined for calculation of mean weight. Missing values indicate fish were not collected at that station.

Location of Collection	1982		1983	
	weight (g)	sample size	weight (g)	sample size
Leavenworth NFH	19.5	61	18.8	33
Rocky Reach Dam (Rkm 763)	----	--	18.4	20
Priest Rapids Dam (Rkm 634)	----	--	19.8	20
McNary Dam (Rkm 467)	24.8	55	21.0	40
John Day Dam (Rkm 347)	23.6	60	----	--
Bonneville Dam (Rkm 232)	24.8	24	22.3	24
Jones Beach (Rkm 75)	26.0	55	22.7	14

Appendix 1E.

a). **Analysis of variance for mean body weight (g) of two hatchery release groups of spring chinook salmon smolts sampled at six locations during the 1983 seaward migration. Asterisk** (*) denotes **P<0.05.**

Source	df	MS	F-ratio
Station	5	80.94	2.71 *
Release group	1	65.78	2.21
Station x release	5	25.39	0.85
Error	**139**	**29.81**	

b). **Analysis of variance for mean body weight (g) of four hatchery release groups of spring chinook salmon smolts sampled at five locations during the 1982 seaward migration. Asterisk (*) denotes** P<0.05.

Source	df	MS	F-ratio
Station	4	391.44	8.04 *
Release group	3	14.58	0.29
Station x release	**12**	36.35	0.74
Error	235	48.66	

Appendix 2. Analysis of variance tables and Newman-Keuls tests for data used to estimate daily food consumption and caloric intake (C) of spring chinook salmon smolts at selected locations during seaward migration in the Columbia River.

Appendix 2A.

a). Analysis of variance of mean dry weight of stomach contents (after $\log_e (x + 1)$ transformation) from spring chinook salmon smolts collected at 4-hour intervals over a 24-hour period in the Columbia River near Arlington, Oregon, 1982. Asterisk (*) denotes $P<0.05$.

Source	df	MS	F-ratio
Between time periods	5	7.98	5.897 *
Within time periods	112	1.35	
Total	117		

b). Newman-Keuls test for mean stomach contents (mg dry weight) from fish collected near Arlington, Oregon, 1982. Underlined values are not significantly different ($P>0.05$).

	Day			Night		
Time	1300	0500	0900	1700	0200	2100
Mean stomach content (mg food dry wt.)	21.0	24.5	31.1	41.8	47.7	72.0

Appendix 28.

a). Analysis of variance of mean stomach contents (dry weight) from spring chinook salmon smolts collected at 4-hour intervals over a 24-hour period in the Columbia River at Crescent Bar, Washington, 1983. Asterisk (*) denotes $P<0.05$.

Source	df	MS	F-ratio
Between time periods	5	1457.5	3.572 *
Within time periods	114	408.0	
Total	119		

b). Newman-Keuls test for mean stomach contents (mg dry weight) for fish collected near Crescent Bar, Washington, 1983. Underlined values are not significantly different ($P>0.05$).

Time	0100	0500	0900	1300	1700	2100
Mean stomach content (mg food dry wt.)	13.0	16.6	23.9	28.3	28.9	36.1

Appendix 2C. Analysis of variance of mean stomach contents (dry weight, after log, (x + 1) transformation) from spring chinook salmon smolts collected at 4-hour intervals over a 24-hour period in the Columbia River near Arlington, Oregon, 1983.

Source	df	MS	F-ratio
Between time periods	5	1.16	1.776 NS
Within time periods	112	0.91	
Total	117		

Appendix 2D.

a). Analysis of variance of caloric density (cal/g dry weight) of stomach contents of spring chinook salmon smolts collected at 4-hour intervals over a 24-hour period in the Columbia River near Arlington, Oregon. Data was pooled for 1982 and 1983. Asterisk (*) denotes P<0.05.

Source	dF	MS	F-ratio
Time	5	161776	3.961 *
Year	1	181720	4.450
Time x year	5	40547	0.990
Total	48	40832	

b). Newman-Keuls test for the caloric density (cal/g dry weight) of the stomach contents of spring chinook salmon smolts collected near Arlington, Oregon. Data pooled for 1982 and 1983. Underlined values are not significantly different (P>0.05).

Time	0900	1300	0100	2100	1700	0500
Mean caloric value (cal/g dry wt)	5321	5348	5511	5538	5548	5652

Appendix 2E. Analysis of variance of caloric density (cal/g dry weight) of stomach contents from spring chinook salmon smolts collected from 1700-2100 hours from the Columbia River near Crescent Bar, Washington 1983 and near Arlington, Oregon, 1982 and 1983.

Source	df	MS	F-ratio
Between stations	2	76608.8	1.178 NS
Within stations	12	65022.4	
Total	14		

Appendix 3. Analysis of variance and chi-square analysis tables showing effect of swimming activity and food consumption by smolts on body lipids, growth, and survival in seawater.

Appendix 3A. Analysis of variance for total caloric values of spring chinook salmon subjected swimming to activity (active and inactive) and feeding regime (fed and starved) treatments for 35 days in 1983. Asterisk (*) denotes P<0.05.

a). **Advanced photoperiod experiment**

Source	df	MS	F-ratio
Activity	1	177.15	0.874
Feeding regime	1	6342.59	31.315 *
Activity x feeding	1	270.16	1.333
Error	32	202.54	

b). **Natural photoperiod experiment**

Source	df	MS	F-ratio
Activity	1	168.25	0.785
Feeding regime	1	9346.48	43.653 *
Activity x feeding	1	9.56	0.044
Error	30	214.10	

Appendix 3B. Analysis of variance for percentage of body lipid (arcsine transformed) of spring chinook salmon subjected to swimming activity (active and inactive) and feeding regime (fed and starved) treatments for 35 days in each of two experiments in 1983. Asterisk (*) denotes $P<0.05$.

a). Advanced photoperiod experiment

Source	df	MS	F-ratio
Activity	1	0.02	0.008
Feeding regime	1	148.72	46.761 *
Activity x feeding	1	30.42	9.566 *
Error	32	3.18	

b). Natural photoperiod experiment

Source	df	MS	F-ratio
Activity	1	0.01	0.004
Feeding regime	1	112.20	35.690 *
Activity x feeding	1	2.15	0.686
Error	29	3.14	

Appendix 3C. Chi-square analysis of three-dimensional contingency table of survival of spring chinook salmon in seawater challenge at two levels of swimming activity and two levels of feeding regime. Asterisk (*) denotes $P<0.05$.

Source	df	χ^2
Advanced photoperiod experiment		
Mutual independence	4	38.36 *
Partial independence		
Survival	3	38.36 *
Feeding regime	3	28.48 *
Activity	3	12.60 *
Natural photoperiod experiment		
Mutual independence	4	52.51 *
Partial independence		
Survival	3	52.46 *
Feeding regime	3	52.51 *
Activity	3	4.55
Survival x feeding regime	1	47.61 *

Appendix 4. Analysis of variance and chi-square analysis tables showing effect of high dietary lipid and starvation on subsequent fatty acid content of spring chinook salmon smolts and their survival in seawater.

Appendix 4A. Analysis of variance for arcsine of percent lipid in spring chinook salmon subjected to different dietary lipid levels (12% and 19%), and feeding regime (fed or starved) for 35 days in 1983. Asterisk (*) denotes $P \leq 0.05$.

Source	df	MS	F-ratio
Dietary lipid	1	10.88	3.492
Feeding regime	1	163.81	52.582 *
Lipid x feeding	1	1.16	0.372
Error	27	3.11	

Appendix 4B. Analysis of variance of mean body weight (g) for spring chinook salmon subjected to different dietary lipid levels (12% and 17%) and feeding regimes (fed and starved) for 35 days. Asterisk (*) denotes $P<0.05$.

Source	df	MS	F-ratio
Dietary lipid	1	77.44	1.42
Feeding regime	1	2199.77	40.25 *
Lipid x feeding	1	-48.96	-0.89
Error	26	54.66	

Appendix 4C. Three dimensional chi-square analysis of survival of seawater challenge by juvenile spring chinook salmon at two levels of feeding regime (fed and starved) and two levels of dietary lipid (12% and 17%). Asterisk (*) denotes $P \leq 0.05$.

Source	df	X^2
Mutual independence	4	35.89 *
Partial independence		
Survival	3	36.27 *
Feeding regime	3	31.37 *
Dietary lipid	3	10.08 *